EVOLUTION IN A TROPICAL ARCHIPELAGO: COMPARISONS WITHIN AND AMONG 50 SPECIES OF PHILIPPINE BIRDS

By

Kyle K. Campbell

RECOMMENDED:

Dr. Kevin McCracken

Dr. Naoki Takebayashi

Dr. Kevin Winker, Advisory Committee Chair

Dr. Diane Wagner
Chair, Department of Biology and Wildlife

APPROVED:

Dr. Paul Layer
Dean, College of Natural Science and Mathematics

Dr. John C. Eichelberger
Dean of the Graduate School

11/28/17
Date

EVOLUTION IN A TROPICAL ARCHIPELAGO: COMPARISONS WITHIN AND AMONG 50 SPECIES OF PHILIPPINE BIRDS

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Kyle K. Campbell, B. S.

Fairbanks, Alaska

December 2013

Abstract

Tropical islands are hotspots for speciation and the generation of genetic diversity within species. The islands of the Philippines have experienced dynamic biogeographic histories, making them excellent for studying the processes affecting the generation and distribution of genetic diversity. I tested hypotheses about the distribution of population genetic diversity within species and examined levels of genetic and phenotypic divergence within 50 species of Philippine birds. The first study empirically tested two models for the distribution of population genetic diversity (increasing diversity with decreasing latitude and decreasing diversity approaching range edges) and found no support for either model. This was not unexpected; in tropical island systems populations are fragmented and potentially more isolated, and their genetic diversity can be limited by island size. However, island size was not correlated with population genetic diversity. Instead, estimates of time spent in evolutionary isolation, inferred from genetic distances between populations within species, were positively correlated with population genetic diversity. This result suggests a possible link between the generation of genetic diversity within populations and the generation of new evolutionary lineages in this system. The second study measured and compared genetic and phenotypic divergence between 136 populations within 50 species and tested the prediction that avian diversity is underestimated in the Philippines. More than half of these species included at least one population that exceeded species-level divergence under conservative thresholds. These results support an urgent need for the taxonomic revision of Philippine birds and have implications for the management of biodiversity in island systems.

Table of Contents

Page

List of Figures

Page

List of Tables

Page

List of Supplementary Materials

Page

List of Appendices

Page

Acknowledgments

This research was supported by the U. S. Department of Agriculture, John DuPont, the Eurasian Pacific Wildlife Foundation, and the Friends of Ornithology. This research was conducted under the supervision of Dr. Kevin Winker. For the specimens used in this study, I thank the Department of Environment and Natural Resources of the Philippines (DENR), including the Protected Areas and Wildlife Bureau and the Protected Areas and Wildlife Divisions from Region 7 for permitting and project assistance. A special thanks also goes to collaborators at the National Museum of the Philippines, including Virgilio Palpal-latoc, Rolly Urriza, and Directors Gabriel Casal and Corazon Alvina, and to Edwin Cedella, Almeo Bontigao, and many others for their invaluable assistance in the field. I thank Herman Mays of the Cincinnati Museum of Natural History and Bob Kennedy for lending tissue samples. Lastly, I thank Tom Braile for his fundamental assistance in setting up field efforts.

General Introduction

Tropical island systems have greatly influenced our understanding of evolution since the beginning of evolutionary biology (Wallace 1858, Darwin 1859). Isolation, as best evidenced by island populations, can lead to adaptation to local environments, and through mechanisms of selection and genetic drift island populations diverge from mainland populations (MacArthur & Wilson 1967). As a result of this, endemism is commonplace on tropical islands. Although representing only 5% of the world's landmass, islands account for as much 20% of global biodiversity (TIB Partners 2012). Island systems are excellent for studying the processes of divergence and speciation, and the factors that generate this diversity.

Islands are not only hotspots for speciation; they are also hotspots for extinction. Island endemic populations are often small and vulnerable to outside forces, as a result of this island species account for up to 80% of extinctions worldwide over the past 500 years (TIB Partners 2012). Conservation efforts focus heavily on island species, yet much remains unknown about diversity and evolution in island systems. Conservation planning is hindered by a deficiency of data on divergence within species in many island systems. Continuing research on island populations is important for conservation planning.

The ~7,100 islands of the Philippines host a high diversity of endemic plants and animals. Myers *et al.* (2000) considered the Philippines to be one of the world's 'hottest' biodiversity hotspots, where a third of the 600 endemic vertebrate species are threatened by extinction (Heaney & Regalado 1998, IUCN 2013). With only 3% of primary vegetation cover remaining in the country, anthropogenic forces threaten the survival of

not just species but whole communities (Myers *et al.* 2000). Birds have on average fewer endemic species than other terrestrial vertebrates in the Philippines (Myers *et al.* 2000). Unlike most vertebrates, birds are volant and have exceptional dispersal capabilities. This is evidenced by the 160 or more migrant bird species that travel annually from the Philippines to higher latitudes to breed, including Nearctic breeders such as Ruddy Turnstone (*Arenaria interpres*) and Arctic Warbler (*Phylloscopus borealis*; Kennedy *et al.* 2000).

However, for some species of Philippine birds overwater dispersal is probably a serious barrier to gene flow (Diamond & Gilpin 1983, Jones & Kennedy 2008). The majority of more widespread, non-endemic bird species (80%) are represented by endemic subspecies in the Philippines (Dickinson *et al.* 1991). Because taxonomy relies heavily on phenotypic characters, cryptic island populations may be overlooked or misclassified as subspecies despite large genetic divergence. Species-level endemism is probably underrepresented in Philippine birds (Peterson 2006, Lohman *et al*. 2010). Identifying which populations merit species recognition requires careful consideration of both genetic and phenotypic characters (Winker 2009).

A complex geological history and multiple colonization events make the birds of the Philippines an excellent system in which to study the factors affecting diversity within and among populations. The Philippines represent four distinct faunal regions that have never been in geological contact, each hosting their own endemic species and subspecies (Dickinson *et al.* 1991). Many Philippine birds have biogeographic origins in Borneo, other islands of the Sunda Shelf, and Southeast Asia, although Australasian

influences from the South and East Asian influences from the north are apparent in different faunal regions (Jones & Kennedy 2008). Recently, elevations of subspecies to full biological species have been proposed for Philippine birds based on cryptic phenotypic divergence (Collar 2011, Rasmussen *et al.* 2012) and genetic divergence (Sheldon *et al.* 2009, 2012, Oliveros *et al.* 2011).

Here I seek to answer questions about the relationships between population-level genetic diversity and factors such as geography, taxonomy, and isolation within multiple species of Philippine birds, and to assess levels of genetic and phenotypic divergence among populations within species and to identify populations that merit taxonomic revision. In my first chapter, I compare distributions of within-species population genetic diversity among nine species of Philippine birds, and test for effects from latitude, island size, and evolutionary isolation on the distribution of this diversity. In my second chapter, I measure genetic distances between pairs of populations within 50 species of Philippine birds, and compare genetic distances with quantitative measures of phenotypic divergence, finding high levels of diversity among some Philippine birds that is not recognized by current taxonomy. This research is relevant for taxonomists, conservationists, and evolutionary biologists interested in the processes that cause biological diversity.

Chapter 1: Evolutionary isolation is correlated with population genetic diversity in nine Philippine bird species. [1]

1.1 Abstract

Distributions of population genetic diversity inform both the management of biodiversity and our understanding of the evolutionary processes that generate it. Although population genetic diversity is often negatively correlated with latitude, other patterns have been observed (e.g., central-peripheral and island effects). Few studies have focused exclusively on patterns of population genetic diversity in tropical island systems, where populations are fragmented and their genetic diversity can be limited by island size. We tested three hypotheses about the distribution of within-species genetic variation (latitudinal, central-peripheral, and random) among 3-4 populations each of nine species of sedentary birds whose ranges encompass all or most of the Philippines (5° to 18° N). Population genetic variation was estimated using mtDNA sequence data (ND2). Overall, distributions of genetic diversity were highly variable. Results showed no support for either a model of increasing diversity with decreasing latitude or a central-peripheral model for the distribution of avian population genetic variation. Island size, which may affect our results through a relationship with effective population size, was not correlated with population genetic diversity. However, estimates of time spent in evolutionary isolation, for populations within species, were correlated with population genetic diversity. This unexpected result suggests that there is a link in this system between two

[1] Campbell, K. and K. Winker. 2013. Evolutionary isolation is correlated with population genetic diversity in nine Philippine bird species. Submitted to *Ecology Letters*.

sets of processes usually considered separately, the generation of genetic diversity within populations and the generation of new evolutionary lineages (speciation). Although further work is needed to understand why evolutionary isolation in this system affects population genetic variation more than island size or latitude, our results have important implications for evolutionary biology and for managing biodiversity in island systems.

1.2 Introduction

One of the most well supported patterns in the distribution of biological diversity is the increase in species diversity as latitude decreases (Fischer 1960, Rosenzweig 1995), and it has been shown that this pattern can also hold true for genetic diversity within populations (Martin & McKay 2004, Hughes & Hughes 2007, Adams & Hadley 2012). The increase in species diversity towards the equator may result from the differential availability of energy driving higher rates of evolution at low latitudes and higher extinction rates nearer the poles (Gaston & Blackburn 1996, Hillebrand 2004, Mittelbach *et al*. 2007), and population genetic diversity may be affected in a similar way (Vellend 2003, Vellend & Geber 2005). However, in a recent study of a Neotropical assemblage of birds, Miller *et al.* (2010) found that population genetic diversity did not increase with decreasing latitude, in contrast to the distribution of species richness. Instead, Miller *et al.* (2010) found a central-peripheral model to best describe the distribution of population genetic variation, in which the greatest variation was typically found at the midpoint of species' ranges.

The central-peripheral model is predicted by the mid-domain effect (Colwell & Lees 2000), wherein edge populations are unlikely to experience the highest levels of diversity due to geometric constraints on dispersal (i.e., central populations may experience more immigration and gene flow than edge populations). Although this model may be appropriate in many mainland systems where gene flow between populations is common (Eckert *et al.* 2008), it may not be applicable in systems in which species' distributions are highly fragmented, such as among tropical islands. Within species, genetic variation is related to population size, and in island systems, where gene flow is often limited between islands, genetic divergence among island populations and genetic diversity within these populations are both affected by island size, probably due to the stronger effect of genetic drift on islands with small effective population sizes (Frankham 1996, 1997, Woolfit & Bromham 2005). Because island taxa are likely to experience less gene flow among populations, and because population sizes are often small on islands, island systems may depart from the patterns of population genetic diversity observed in mainland systems.

In the Philippines, bird populations are distributed across more than 7,000 islands ranging in size from less than 10 km^2 to more than 100,000 km^2, and species ranges in the Philippines are likely to have been relatively stable over time, having experienced little Pleistocene fluctuation (Jones & Kennedy 2008). The geological and biogeographic history of the Philippines, and the exceptional dispersal abilities of volant animals like birds, make this an excellent system in which to test both the latitudinal gradient and central-peripheral models of the distribution of population genetic diversity. Here we

examine the distribution of population genetic variation along a latitudinal transect of nine species of Philippine birds from eight families and three orders.

1.3 Materials and methods

In the Philippines, over 570 species of migratory and sedentary birds are distributed across a tropical archipelago stretching latitudinally along the Western Pacific Rim (Kennedy *et al*. 2000; Fig. 1.1). Most of the islands of the Philippines can be separated into three distinct faunal regions: the Luzon faunal region (18-12° N), with its largest island being Luzon (109,965 km^2); the Visayas (12-9° N), with its largest islands being Negros (13,704 km^2; not sampled) and Panay (12,011 km^2); and the Mindanao faunal region (8-5° N), with its largest island being Mindanao (at 97,530 km^2; Heaney 1986). A fourth faunal region, the islands associated with Palawan, are connected to the Sunda Shelf and therefore share biogeographic histories with Borneo and mainland Southeast Asia; populations from the islands of this last faunal region were not included in this study.

We selected nine species (of three orders and eight families) of sedentary (nonmigratory) birds whose ranges encompass most of the Philippine archipelago. These choices were based on the availability of population-level samples from vouchered museum specimens (Table S1.1). For each species, we sampled 4-10 individuals from each of three to four latitudinally separated islands (Table 1.1; see Table S1.1 for specific localities). Variable sample sizes were avoided when possible, however for some populations sample size was limited by specimen availability. Although we were careful not to knowingly sample across distinct populations within islands, cryptic diversity is

abundant in the Philippines (Sheldon *et al.* 2009) and unknowingly sampling across cryptically divergent populations within islands could still occur.

We sequenced the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) from 28 populations (233 individuals) from these nine species. DNA was extracted from frozen tissue (or temporarily ethanol preserved for borrowed samples) and amplified using standard PCR protocols with the forward primer L5215 (Hackett 1996) and reverse primer H6313 (Johnson & Sorenson 1998). Sequencing was performed by the University of Washington's High Throughput Genomics lab (www.htseq.org) using the same primers and the following custom internal forward primers designed for this study: Eumy.ND2inter (ACAAAAACCCCAGCACTWAG), Hypsi.ND2inter (TAAACTCAATCAAAACCCTA), OtusND2inter (CCCAACCCTATTGACCMYAA), ParusND2inter (TTCTCCTCCATCTCCCACCT), Phapitre.ND2inter (CTACTAACCTTCTAYCTWTA), SittaND2inter (TATTAACCACCATAGCCATC), and Zoster.ND2inter (CTACTCACATGCATAGCCGT). Sequence data were cleaned and aligned using Sequencher 4.0. The analyzed ND2 sequence datasets ranged in length from 899 to 1029 bp.

To assess the distribution of within-species genetic variation, we compared nucleotide diversity per site (π) among three latitudinally separated island populations for most species. In one species, *Phapitreron leucotis*, we sampled four island populations. Estimates of π were generated using DnaSP (Librado & Rozas 2009). The latitudinal gradient model would produce an increase in diversity with decreasing latitude, wherein the expression $\pi_{i+1} - \pi_i$ would have non-negative results more often than predicted by

chance, where π_i represents the population sampled from the highest latitude for a given species, and π_{i+1} is population from the next highest latitude (Martin & McKay 2004). The central-peripheral model, as observed by Miller *et al*. (2010) in Neotropical birds, would produce a humped distribution in which the largest value of π is found in mid-range rather than edge populations. Our null hypothesis considers that the highest within-species value of π will occur randomly among populations; thus, by chance, the mid-range should show a species' peak in 1/3 of cases, and the expression $\pi_{i+1} - \pi_i$ should be non-negative with a frequency of 0.5.

To analyze population structure and levels of divergence among populations, we generated haplotype networks for each species using DnaSP v5, Network 4.6.1.0, and performed pairwise calculations of genetic divergence between populations using DnaSP and MEGA version 5 (Librado & Rozas 2009, Bandelt *et al*. 1999, Tamura *et al.* 2011). We inferred estimated effective population size for each population sampled using the mutation parameter θ (Theta-W) (Watterson 1975) calculated in DnaSP (Librado & Rozas 2009). We estimated within-species evolutionary isolation for each population from the between-population distance measures D_a and Jukes-Cantor corrected *p*-distance, wherein evolutionary isolation for a given population is equal to the average of distances between that population and all other populations sampled (Supplementary Table S1.2). In addition, we estimated the population demographic parameters Tajima's *D* (1989), Fu's F_s (1997), and Romis-Onsins & Rozas' R_2 (2002) for each of the 29 populations studied in DnaSP (Librado & Rozas 2009). Statistical significance for these parameters was calculated by comparing estimated values to those expected from 50,000

coalescent simulations using a model of constant population size in DnaSP v5 (Librado & Rozas 2009). To test for significant relationships between π and potential driving mechanisms, such as island size and evolutionary isolation, we performed ordinary least squares regressions.

1.4 Results

Among all populations, π varied from 0.00039 to 0.0096 and had a median value of 0.0016 (Table 1.2). Despite variability in values of π among species, there was not a significant species effect (ANOVA, $P = 0.26$). Haplotypes were not shared between populations in any species (Fig. 1.2), though in one species, *Pycnonotus goiavier*, two populations exhibited mtDNA paraphyly. The distribution of population genetic diversity within species was quite variable (Fig. 1.3).

In our test of increasing diversity with decreasing latitude there were 19 opportunities to evaluate the expression: $\pi_{i+1} - \pi_i$. Of these, eleven were non-negative (frequency = 0.58), which is not significantly different from the expected frequency of 0.5 predicted by a null model of random change with respect to latitude (exact binomial test, $P = 0.65$). Corroborating this, a regression of π and latitude showed no significant relationship ($P = 0.5$).

Maximum nucleotide diversity was found in edge populations in seven species (frequency = 0.78). This observation was not significantly different from the expected frequency of 0.66 predicted by a random distribution of diversity (exact binomial test, $P = 0.73$). Maximum values of π were found in a mid-range population in 2 species

(frequency = 0.22), which was also not significantly different from our null expectation of 0.33 (exact binomial test, $P = 0.73$). We thus could not reject our null hypothesis of a random distribution of peak population genetic diversity.

Post-hoc analyses using ordinary least square regressions showed no relationship between population genetic diversity and either island size or species richness ($P = 0.47$ and 0.41, respectively). However, avian species richness was significantly correlated with island size ($P = 0.025$), as expected. Estimates of inferred effective population size (θ) and island size were not significantly correlated ($P = 0.46$, Table S1.3). In two species, *P. leucotis* and *I. philippinus*, we observed the highest levels of diversity in the smallest islands sampled, despite presumably smaller population sizes on these islands (Bohol Island and Panay Island, respectively, Fig. 1.3). In these species there was an apparent correlation between populations with high diversity and those with large genetic divergence among populations, leading us to an *a posteriori* hypothesis that population genetic diversity may be influenced by evolutionary isolation within islands.

Overall, our two estimates of population evolutionary isolation (within species) were significantly correlated with population genetic diversity (ordinary least squares regressions, $P = 0.007$ and 0.018; Fig. 1.4), indicating a positive relationship between population genetic diversity and evolutionary isolation in this system. Assuming no gene flow between populations, and rates of mtDNA mutation between lineages ranging from 2-6% per million years (Lovette 2004, Ho 2007, Ho *et al*. 2011), the number of mutations we observed within each population and from the nearest points of common ancestry between populations corresponded to 0.1 to 6.4 My of evolutionary isolation (median of

0.55 My, average 0.81 My; SD = 0.92 My; Supplementary Table S1.4). Population demographic parameters did not indicate significant population expansion or decline for most populations (Supplementary Table S1.3).

1.5 Discussion

Among the nine species of resident Philippine birds we studied, the distribution of population genetic diversity did not match either the latitudinal gradient model or the central-peripheral model. Given that haplotypes were not shared among populations, however, the latter is not unexpected. The mid-domain model is less likely to apply in systems where gene flow is severely restricted (Miller *et al*. 2010). Similarly, the effect of latitude on population genetic diversity may be less apparent in species with discontinuous ranges (Guo 2012), such as among island systems. Our results demonstrate that distributions of population genetic diversity in tropical island systems can depart from predictions drawn from mainland systems. There are numerous possible reasons for this, but the most important likely stem from principles of island biogeography (MacArthur & Wilson 1967).

Within island populations, genetic diversity arises through mutation and immigration (MacArthur & Wilson 1967), and the extent to which this diversity persists can be dictated largely by genetic drift (Wright 1969). Genetic drift has a stronger effect on smaller populations (Wright 1969), and given that island area can directly affect population size (Frankham 1997, Grant 1998, White & Searle 2007), one can expect that in small islands the effect of drift is greater (MacArthur & Wilson 1967, Hughes &

Hughes 2007). In our study system, edge populations occupy substantially larger islands than mid-range populations, and one might therefore expect island size, through its effect on effective population size (N_e), to influence the observed distribution of population genetic diversity. Although island size appears to affect avian species richness in the Philippines, as island biogeography predicts (MacArthur & Wilson 1967), it does not explain the variation we observed in the distribution of population genetic diversity. Island size may not be a useful surrogate for N_e in this system, but, regardless, our results indicate that population genetic diversity can be independent of island size, and this in itself is an important result.

Unlike observations from other island systems (e.g., Vellend 2003), population genetic diversity and species richness do not appear to be linked in these Philippine birds. We expect that the presence of mountain ranges affects habitat heterogeneity on most of the islands we studied, and this may stimulate greater numbers of species while limiting the area available for populations of habitat specialists to occupy. On the larger islands of Luzon and Mindanao multiple lineages are present for some of the species we studied (*P. elegans, S. oenochlamys, Z. montanus*; described in Dickinson *et al.* 1991 or otherwise identified by Jones & Kennedy 2008), indicating that barriers to gene flow also exist within islands for some species. Habitat fragmentation can also cause barriers to gene flow, and, more recently, anthropogenic forces have severely limited the habitable area available to many species; deforestation and resource extraction threaten most habitat types in the Philippines, and some islands are more heavily impacted than others (Heaney & Regalado 1998).

Although independent estimates of population size are warranted, it may be the case that population size does not affect population genetic diversity in this system. Most of the populations studied appear not to be undergoing population growth or decline (Table S1.3), however significant deviations from a model of constant population size for some populations indicate heterogeneity among the populations studied. Further work will be needed to better understand the relationship between effective population sizes and island size in this system.

Given that we observed no apparent gene flow between populations, based on the absence of shared haplotypes among populations within species (Fig. 1.2), it was plausible that the majority of the variation we observed within populations evolved *de novo* through new mutations. If this were true, populations having experienced longer periods of evolutionary isolation might attain higher levels of genetic diversity. Our results support this hypothesis; evolutionary isolation, as inferred from two different pairwise divergence estimates (D_a and Jukes-Cantor corrected *p*-distance), was significantly correlated with population ND2 nucleotide diversity (Fig. 1.4).

This unexpected result suggests that there is a link in this system between the generation of genetic diversity within populations and the generation of new evolutionary lineages (speciation). Given sufficient time in evolutionary isolation, new variation is generated through mutations. It is possible that the populations we studied may have had such time. Speciation, in most cases, occurs in the absence of gene flow between populations (Coyne & Orr 2004, Price 2008), and in the Philippines many non-migratory birds are believed to be poor overwater dispersers (Diamond & Gilpin 1983). Because of

this, many islands host their own endemic species. Avian endemism may even be underestimated in the Philippines due to an abundance of cryptic diversity (Sheldon *et al.* 2009, Lohman *et al*. 2010, Collar 2011). The absence of gene flow that we observed suggests a scenario in which island populations are evolving independently from one another, and as such they can behave as incipient species subject to different selection pressures and, consequently, different rates of evolution (anagenesis). A scenario such as this could explain both the high degree of variability we observed among species and the relationship between population genetic diversity and evolutionary isolation.

Although independent estimates of time in isolation would be more informative, we were able to estimate the time required for the number of mutations we observed between populations to accrue (Table S1.4). Timing estimates are dependent on the rate of mutation, and there is some disagreement about mtDNA mutation rates in birds (Lovette 2004, Ho 2007, Nabholz *et al.* 2009, Ho *et al.* 2011). However, our estimate of time in evolutionary isolation for the populations we sampled considers that the "standard" substitution rate of 2% per My between lineages derives from interspecific comparisons, and that within species the rate is probably faster and more variable (Ho 2007, Ho *et al*. 2011). We are cognizant of the circularity of comparing these estimates with the numbers of mutations observed; however, our estimates of the time in evolutionary isolation that would be necessary for the number of mutations we observed to accrue (0.1 – 6.4 My) are consistent with geological and biogeographic timescales of modern Philippine islands and their avifauna (Hall 1998, Jones & Kennedy 2008, Vallejo Jr. 2011). In island systems, rates of nonsynonymous mtDNA substitution in birds have

been shown to be higher than in mainland systems (Johnson & Seger 2001), however, actual rates of substitution may vary among the species we studied. Further work is needed to establish why evolutionary isolation may contribute more to population genetic diversity than island size or latitude.

The disparate nature of biogeographic histories among birds in this region (Diamond & Gilpin 1983, Jones & Kennedy 2008, Sheldon *et al.* 2009, Vallejo Jr. 2011, Andersen *et al.* 2013) and the variation in distributions of population genetic diversity observed here suggest that in the Philippines the factors affecting evolution within and among species are complex and multifarious. In the Philippines, anthropogenic forces threaten the survival of not just species but whole communities (Heaney & Regalado 1998). It is important to incorporate genetic analyses into conservation planning, especially in highly imperiled and fragmented populations (Redding & Mooers 2006), as conventional approaches to assessing diversity may oversimplify complicated systems such as the Philippines. Assessing the distribution of genetic diversity within tropical island populations increases our understanding of the processes generating biodiversity and can also inform conservation management in these systems.

1.6 Acknowledgments

Our field effort was supported by the U. S. Department of Agriculture, John DuPont, the Eurasian Pacific Wildlife Foundation, and the Friends of Ornithology. We thank the Department of Environment and Natural Resources of the Philippines (DENR), including the Protected Areas and Wildlife Bureau and the Protected Areas and Wildlife Divisions from Region 7 for permitting and project assistance. We also thank Director Pollisco, Director Mundita Lim, Carlo Custodio, Anson Tagtag, Director Augustus Momongan, Reynaldo Yray, Division chief Kho, Gloria Dawson, and many others who participated in this project. A special thanks also goes to our collaborators at the National Museum of the Philippines, including Virgilio Palpal-latoc, Rolly Urriza, and Directors Gabriel Casal and Corazon Alvina, and to Edwin Cedella, Almeo Bontigao, and many others for their invaluable assistance in the field. We thank Herman Mays of the Cincinnati Museum of Natural History and Bob Kennedy for lending tissue samples. Lastly, we thank Tom Braile for his fundamental assistance in setting up field efforts.

1.7 References

Adams, R. I. & Hadley, E. A. (2012). Genetic diversity within vertebrate species is greater at lower latitudes. *Evol. Ecol*., 27, 133-143.

Andersen, M. J., Oliveros, C. H., Filardi, C. E., & Moyle, R. G. (2013). Phylogeography of the Variable Dwarf-Kingfisher *Ceyx lepidus* (Aves: Alcedinidae) Inferred from Mitochondrial and Nuclear DNA Sequences. *Auk*, 130, 118-131.

Bandelt, H.J., Forster, P. & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol*., 16, 37-48.

Collar, N.J. (2011). Species limits in some Philippine birds including the Greater Flameback *Chrysocolaptes lucidus. Forktail*, 27, 29-38.

Colwell, R. & Lees, D. (2000). The mid-domain effect: geometric constraints on the geography of species richness. *TREE*, 15, 70–76.

Coyne, J. A., & Orr, H. A. (2004). *Speciation.* Sunderland, MA: Sinauer Associates.

Diamond, J.M., and Gilpin, M.E. (1983). Biogeographic umbilici and the origin of the Philippine avifauna. *Oikos*, 41, 307-321.

Dickinson, E. C., Kennedy, R. S. & Parkes, K. C. (1991). *The birds of the Philippines: an annotated check-list* (Vol. 12). Tring: British Ornithologists' Union.

Eckert, C.G., Samis, K.E. & Lougheed, S.C. (2008). Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Mol. Ecol*., 17, 1170-1188.

Fischer, A.G. (1960). Latitudinal variation in organic diversity. *Evol.,* 14, 64-81.

Frankham, R. (1996). Relationship of genetic variation to population size in wildlife. *Cons. Biol.*, 10, 1500-1508.

Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity*, 78, 311-327.

Gaston, K. J. & Blackburn, T. M. (1996). The tropics as a museum of biological diversity: an analysis of the New World avifauna. *Proc. R. Soc. Lond. B,* 263, 63-68.

Grant, P. R. (1998). *Evolution on Islands*. Oxford Univ. Press, Oxford.

Guo, Q. (2012). Incorporating latitudinal and central–marginal trends in assessing genetic variation across species ranges. *Mol. Ecol.,* 21, 5396-5403.

Hackett, S. J. (1996). Molecular phylogenetics and biogeography of tanagers in the genus Ramphocelus (Aves). *Mol. Phylog. Evol.,* 5, 368-382.

Hall R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall R., Holloway J.D. (eds.) *Biogeography and Evolution of SE Asia.* Backhuys Publisher, Leiden: 99-131.

Heaney, L.R. (1986). Biogeography of mammals in SE Asia: estimates of of rates of colonization, extinction, and speciation. *Bio. Jour. Linn. Soc*., 28, 127-165.

Heaney, L.R. & Regalado, Jr., J.C. (1998). *Vanishing treasures of the Philippine rainforest*. The Field Museum, Chicago.

Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *Am. Nat*., 163, 192-211.

Ho, S. Y. (2007). Calibrating molecular estimates of substitution rates and divergence times in birds. *J. Avian Biol.*, 38, 409-414.

Ho, S. Y., Lanfear, R., Bromham, L., Phillips, M. J., Soubrier, J., Rodrigo, A. G., & Cooper, A. (2011). Time‐dependent rates of molecular evolution. *Mol. Ecol.,* 20, 3087-3101.

Hughes, A.L. & Hughes, M.A.K. (2007). Coding sequence polymorphism in avian mitochondria genomes reflects populations histories. *Mol. Ecol.,* 16, 1369-1376.

Johnson, K. P. & Seger, J. (2001). Elevated rates of nonsynonymous substitution in island birds. *Mol. Biol. Evol.*, 18, 874-881.

Johnson, K. P. & Sorenson, G. D. (1998). Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome b and ND2) in the dabbling ducks (tribe: Anatini). *Mol. Phylog. Evol*., 10, 82-94.

Jones, A. & Kennedy, R. (2008). Evolution in a tropical archipelago: comparative phylogeography of Philippine fauna and flora reveals complex patterns of colonization and diversification. *Biol. Jo. Linn, Soc*., 95, 620-639.

Kennedy, R., Gonzalez, P., Dickinson, E., Miranda, H. Jr. &Fisher, T. (2000). *A guide to the birds of the Philippines*. Oxford: Oxford University Press.

Librado, P. & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451-1452.

Lohman, D. J., Ingram, K. K., Prawiradilaga, D. M., Winker, K., Sheldon, F. H., Moyle, R. G., Ng, P. K. L., Ong, P. S., Wang, L. K., Braile, T. M., Astuti, D., and Meier, R. (2010). Cryptic genetic diversity in "widespread" Southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. *Biol. Conserv*., 143, 1885-1890.

Lovette, I.J. (2004). Mitochondrial dating and mixed-support for the "2% rule" in birds. *Auk*, 121, 1-6.

MacArthur, R. H. & Wilson, E. O. (1967). *The theory of island biogeography*, Vol. 1. Princeton University Press.

Martin, P. R. & McKay, J. K. (2004). Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evol*., 58, 938-945.

Miller, M.J., Bermingham, E., Klicka, J., Escalante, P. & Winker, K. (2010). Neotropical birds show humped distribution of within-population genetic diversity along a latitudinal transect. *Ecol. Lett*., 13, 576-586.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Boren, J.M., Bush, M.B. *et al*. (2007). Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol. Lett*., 10, 315-331.

Nabholz, B., Glémin, S., & Galtier, N. (2009). The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evol. Biol.*, 9, 54.

Network 4.6.1.0., released 30 December 2011, expiry date 1 January 2013. Network. Copyright Fluxus Technology Ltd. 1999-2012. http://www.fluxusengineering.com/

Price, T. (2008). *Speciation in birds*. Greenwood Village, CO. Roberts and Company.

Redding, D.W. & Mooers, A.O. (2006). Incorporating evolutionary measures into conservation prioritization. *Conserv. Biol*., 20, 1670–1678.

Rosenzweig, M. L. (1995). *Species diversity in space and time*. Cambridge Univ. Press, Cambridge, U.K.

Sheldon, F. H., Lohman, D. J., Lim, H. C., Zou, F., Goodman, S. M., Prawiradilaga, D. M., Winker, K., Braile, T. M. & Moyle, R. G.. (2009). Phylogeography of the magpie-robin species complex (Aves: Turdidae: Copsychus) reveals a Philippine species and novel dispersal patterns in the Indian Ocean and S. E. Asia. *J. Biogeo*., 36,1070-1083.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., 28, 2731-2739.

Vallejo Jr, B. (2011). The Philippines in Wallacea*. Biodiversity, Biogeography and Nature Conservation in Wallacea and New Guinea*, 1, 1-27.

Vellend, M. (2003). Island Biogeography of Genes and Species. *Am. Nat.*, 162, 358-365.

Vellend, M. & Geber, M. (2005). Connections between species diversity and genetic diversity. *Ecol. Lett.,* 8, 767-781.

Watterson, G. A. (1975). On the number of segregating sites in genetical models without recombination. *Theor. Popul. Biol.*, 7, 256-276.

White, T. A. & Searle, J. B. (2007). Genetic diversity and population size: island populations of the common shrew, *Sorex araneus*. *Mol. Ecol.,* 16, 2005-2016.

Woolfit, M. & Bromham, L. (2005). Population size and molecular evolution on islands. *Proc. R. Soc. Lond. B*, 272, 2277-2282.

Wright, S. (1969). *Evolution and the Genetics of Populations*, Vol. 2. University of Chicago Press, Chicago.

Table 1.1. Island area, latitude, and avian species richness for all islands sampled. Species richness estimates were derived from species accounts in Dickinson et al. (1991) for most islands. Olango Island species richness is reported from the Olango Island Wildlife Sanctuary (www.olangowildlifesanctuary.org).

Island	**Island Area (**km^2**)**	**Latitude**	**Avian Species Richness**
Luzon	109,965	18º-12ºN	243
Panay	12,011	11º-10ºN	125
Olango	10.3	10ºN	49
Bohol	3,821	9ºN	141
Mindanao	97,530	8º-5ºN	244

Table 1.2. Estimated nucleotide diversity for nine species (28 populations) of Philippine birds. Species are separated by island. Islands are listed in order of decreasing latitude. *n* = individuals sampled, Num Hap = number of haplotypes identified, *H* = haplotypic diversity, π = ND2 nucleotide diversity, and σπ = standard deviation of π. The highest value of π for each species is in bold.

Scientific Name	***n***	**Num Hap**	H	π	σπ
Phapitreron leucotis					
Luzon Island	10	4	0.533	0.00078	0.00033
Panay Island	4	2	0.5	0.00097	0.00052
Bohol Island	4	3	0.833	**0.00162**	0.00043
Mindanao Island	10	3	0.378	0.00039	0.0002
Otus megalotis					
Luzon Island	8	7	0.964	**0.00588**	0.00185
Panay Island	5	5	1	0.00411	0.001
Mindanao Island	9	5	0.722	0.00109	0.00036
Parus elegans					
Luzon Island	10	5	0.844	0.00156	0.00024
Panay Island	10	3	0.511	0.00212	0.00108
Mindanao Island	10	7	0.911	**0.0096**	0.0018
Pycnonotus goiavier					
Luzon Island	10	4	0.733	0.00212	0.00032
Olango Island	9	2	0.222	0.00165	0.00123
Mindanao Island	10	6	0.911	**0.00666**	0.00189
Ixos philippinus					
Luzon Island	10	9	0.978	0.00473	0.00081
Panay Island	10	8	0.955	**0.00619**	0.00088
Mindanao Island	10	8	0.933	0.00216	0.00043
Eumyias panayensis					
Luzon Island	9	3	0.556	**0.00068**	0.00024
Panay Island	10	3	0.378	0.00044	0.00023
Mindanao Island	10	5	0.667	0.00201	0.00101
Ficedula hyperythra					
Luzon Island	9	4	0.694	**0.00124**	0.00038
Panay Island	10	4	0.644	0.00076	0.00023
Mindanao Island	10	3	0.644	0.00076	0.00017
Sitta oenochlamys					
Luzon Island	9	5	0.833	**0.0024**	0.00067
Panay Island	4	3	0.833	0.001	0.00034
Mindanao Island	5	5	1	0.0022	0.00042
Zosterops montanus					
Luzon Island	5	2	0.4	0.00083	0.00049
Panay Island	8	4	0.75	0.00112	0.00027
Mindanao Island	5	5	1	**0.00438**	0.00099

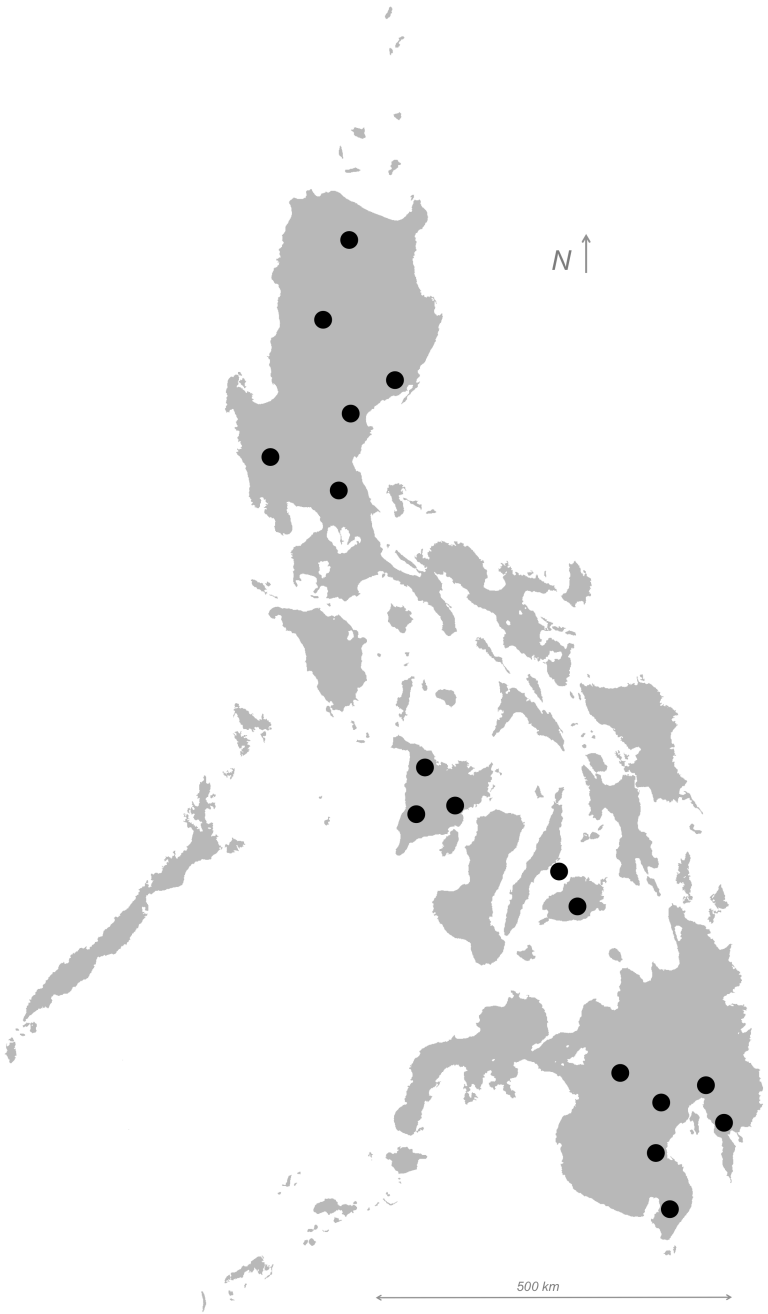


Figure 1.1. Map of the Philippines showing sampling localities for populations used in this study. Islands sampled are, from the northernmost southward: Luzon, Panay, Olango, Bohol, and Mindanao. Table S1.1 provides detailed sampling localities.

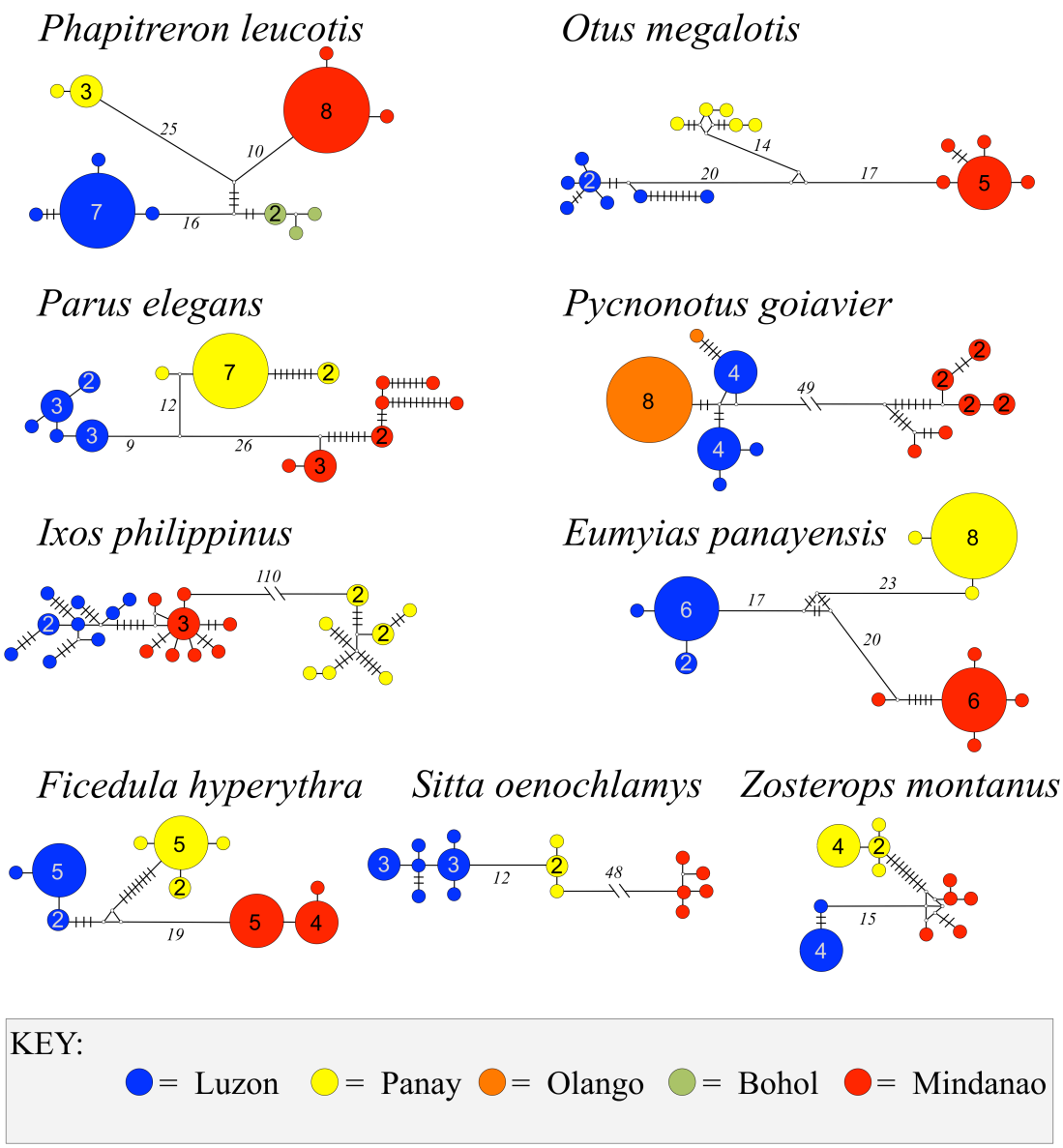


Figure 1.2. Median-joining haplotype networks for nine species (28 populations) of Philippine birds sampled across 3-4 island populations. Small white circles indicate inferred ancestral haplotypes, and the number of nucleotide mutations between haplotypes is given for all branch lengths greater than 1. The number of individuals per haplotype is given for all haplotypes with more than one individual.

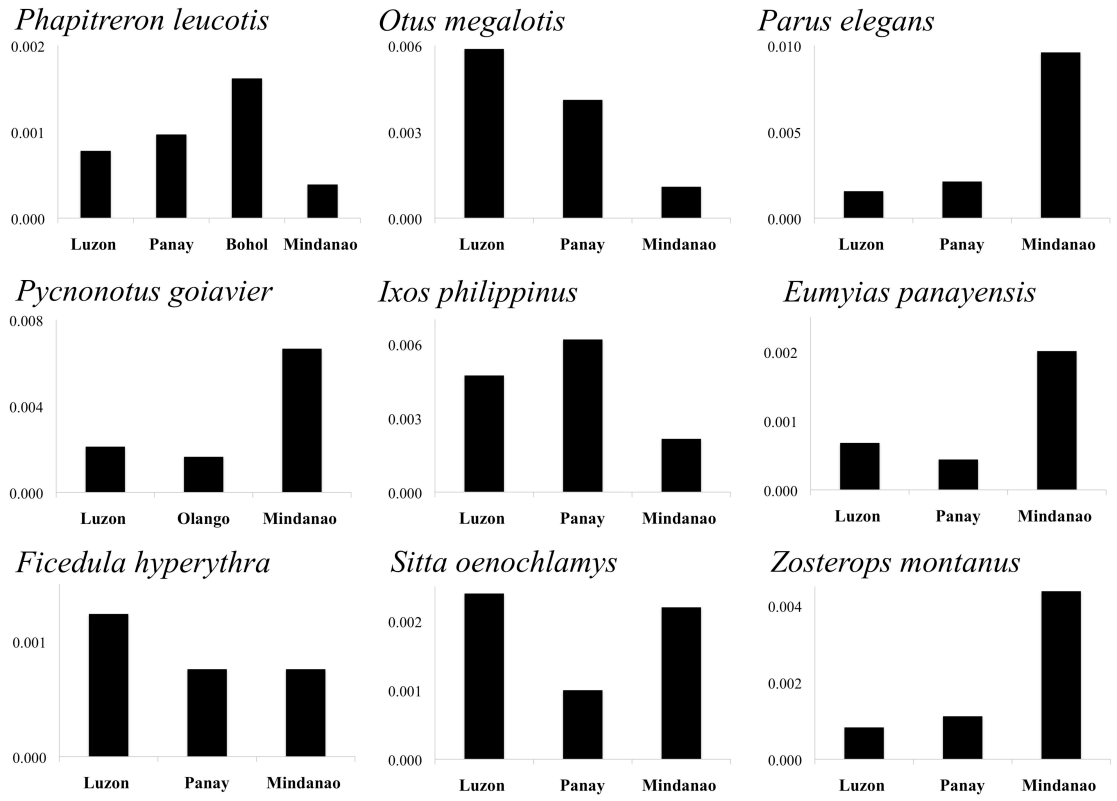


Figure 1.3. Estimated ND2 nucleotide diversity (π) for nine species (28 populations) of Philippine birds sampled across 3-4 island populations. Island populations are presented in order of decreasing latitude for each species.

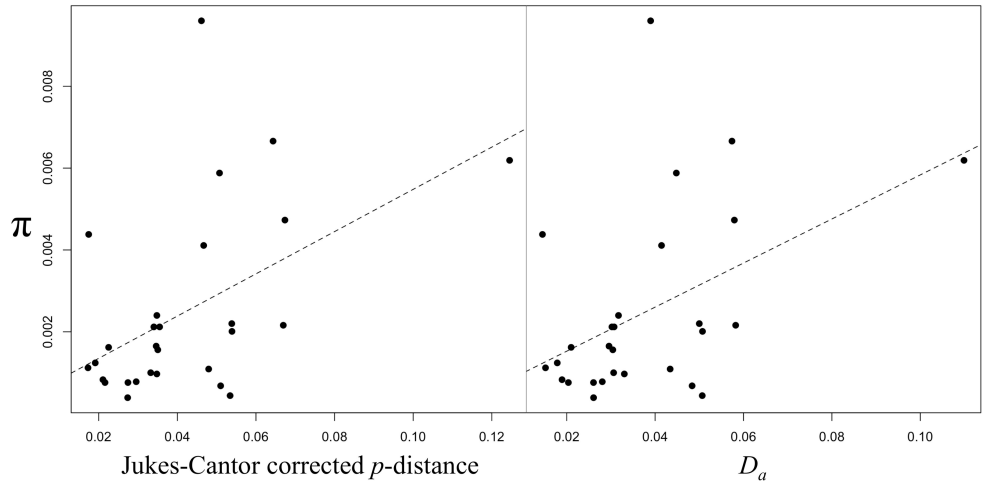


Figure 1.4. Ordinary least squares regressions of π versus estimates of evolutionary isolation for each population. Evolutionary isolation was inferred from pairwise estimates of Jukes-Cantor corrected *p*-distance and D_a between populations (see Supplementary Table S1.2).

1.8 Supplementary Materials

Table S1.1. List of specimens used in this study including taxon identification, island, locality, field catalog numbers, museum voucher numbers, and GenBank accession numbers for ND2 sequences generated in this study. Specific locality information is available upon request from the authors.
(see attached supporting materials)

Table S1.2. Conversion worksheet for estimating within species evolutionary isolation from pair-wise estimates of Jukes-Cantor Corrected *p*-distances and D_a.
(see attached supporting materials)

Table S1.3. Population statistics and results of ordinary least squares regressions, and population demographic parameters.
(see attached supporting materials)

Table S1.4. Conversion worksheet for estimating the amount of time in evolutionary isolation required for numbers of observed mutations to arise between populations.
(see attached supporting materials)

Chapter 2: Genetic and phenotypic divergences in 50 species of Philippine birds show heterogeneous speciation processes and a need for taxonomic revision.[1]

2.1 Abstract

The Philippines are one of the most biologically diverse island groups in the world. Current taxonomy, however, may underestimate levels of avian diversity and endemism in these islands. Although species limits can be difficult to determine among allopatric populations, quantitative methods for comparing phenotypic and genotypic data can identify populations that merit consideration for elevation to species status. Pairwise comparisons of genetic and phenotypic divergence between 136 populations among 50 species indicated as many as 70 populations from 35 or more pairwise comparisons approaching or surpassing species-level divergence under conservative conceptual speciation thresholds. Genetic and/or phenotypic divergences within over half of the species studied were greater than or equal to that observed between full species in other systems. Although broader genetic, phenotypic, and numeric sampling is needed to accurately assess species-level diversity in these taxa, our results support the need for substantial taxonomic revisions among Philippine birds. The conservation implications are profound.

[1] Campbell, K. and K. Winker. 2013. Genetic and phenotypic divergences in 50 species of Philippine birds show heterogeneous speciation processes and a need for taxonomic revision. Prepared for submission to *The Auk*.

2.2 Introduction

Despite difficulties of species delineation, species-level diversity is arguably the most important measure of biodiversity (Mayr 1963, Hurlbert 1971). Most of the world's terrestrial vertebrate diversity has been described to the species level, and species recognition is important in conservation efforts and public awareness (Dickinson et al. 2004, Wilson and Reeder 2005, Frost 2009). In the tropics, where species richness is highest and where much undiscovered biological diversity is believed to exist (Stevens 1989, Bradshaw et al. 2008, Joppa et al. 2011), cryptic diversity can be overlooked or obscured by taxonomy that often relies heavily on phenotypic characters (Bickford et al. 2007, Lohman et al. 2010, Sargis et al. 2013). Conventional approaches to partitioning species diversity have relied on divergence exhibited in sympatry, wherein intrinsic barriers to gene flow provide clear evidence of biological incompatibility between populations (Price 2008). Species status for populations diverging in allopatry, such as island taxa, can be more difficult to ascertain (Mayr 1963, Tobias et al. 2010). In many cases, variations among populations not deemed to be species-level in magnitude cause these populations to be described as subspecies (Winker and Haig 2010), and in island systems individual islands often host endemic subspecies of wide-ranging species (MacArthur and Wilson 1967, Dickinson et al. 2004). Island populations are more prone to extinction than mainland populations, and in birds the majority of recent extinctions were of island endemic species (Donald et al. 2010). Island endemism is an important distinction for conservation planning; conservation efforts often focus on island endemics in tropical island systems, and in the Philippines this is especially true (Heaney and

Regalado 1998, Ong et al. 2002). The conservation of island populations can be profoundly affected by inaccurate species delineations (Lohman et al. 2010); therefore accuracy in recognizing island endemic species is an important conservation issue.

The Philippines are described as one of the world's 'hottest' Biodiversity Hotspots, hosting many endemic and threatened species and subspecies (Myers et al. 2000, Conservation International 2008). Only 3% of original primary vegetation cover remains in the Philippines (Myers et al. 2000), where nearly half of all endemic species are threatened with extinction (Conservation International 2008). Many endemic subspecies are already extinct (Dickinson et al. 1991). Thirty percent of Philippine bird species are currently recognized as endemic; however, nearly 80% of non-endemic species include multiple subspecies that are themselves endemic to different islands (Kennedy et al. 2000). Compared with mammals, amphibians, and freshwater fish, Philippine birds have significantly lower levels of endemism (Myers et al. 2000). However, empirical evidence from a genetic study of seven bird species suggested that avian endemism might be greatly underestimated (Lohman et al. 2010). Determining which island endemic populations deserve full species status, and which do not, remains an unresolved issue (Dickinson et al. 1991, Dickinson et al. 2004, Peterson 2006, Collar 2007, Lohman et al. 2010).

A recent method for scoring phenotypic characters among populations provides a reasonable approach for identifying divergent populations that merit species-level elevation (Tobias et al. 2010), and it has been used to support elevation of populations to full biological species status on islands, including the Philippines (Collar 2011,

Rasmussen et al. 2012). This approach uses average scores from comparisons of well-defined species pairs in sympatry that serve as a baseline for determining a species-level threshold of divergence when comparing allopatric populations. However, speciation can also occur in the absence of obvious phenotypic divergence (Price 2008), especially in island systems where populations can evolve in isolation (MacArthur and Wilson 1967). Through the use of genetic markers, such as mtDNA, it is possible to infer rates of gene flow, evolutionary isolation, and time since common ancestry (Avise 1994, 2000). Although it may be tempting to use simple genotypic or phenotypic markers as stand-alone measures of species-level divergence, speciation may not be fully encapsulated (or diagnosed) by these characters alone (Avise 2000, Price 2008).

Speciation is an inherently multidimensional process (Winker 2009); taxonomy based solely on phenotypic differences, or on stochastic genetic variation, reflects only one dimension of divergence between populations. It is also a discipline that creates taxonomic bins to describe a continuous process. Recognizing the challenges of accurately determining the nature of diversity around the taxonomic "bin" of the species level, it is appropriate to adopt an approach that integrates both phenotypic and genotypic lines of inference. Through integration of genetic and phenotypic datasets, not only can the need for taxonomic revision be assessed, coupling these data might help us understand how some taxa may diverge along different routes to speciation, e.g., remaining phenotypically cryptic despite deep genetic divergence, or, conversely, being dramatically different phenotypically despite shallow genetic divergence (Bickford et al. 2007, Winker 2009). When considering divergences between populations along two axes

(genetic and phenotypic divergence), three principal routes toward speciation are apparent: populations diverging both genetically and phenotypically, populations with divergent phenotypes and shallow genetic structure, and populations with deep genetic divergence despite similar phenotype. Which route taxa diverge along can depend on many factors, including geography, phenotypic plasticity, genetic drift, and the extent and direction of selection (Winker 2009). Here we take such an approach, examining within-species pairwise genetic and phenotypic divergences among populations of 50 species of Philippine birds. Our data enable comparisons among taxa within a multidimensional process space in which multiple routes to speciation exist. In such a process space, populations diverging equivalently along both genetic and phenotypic axes represent one route to speciation, and populations diverging more along one axis than the other represent two other routes (Winker 2009).

2.3 Methods

2.3.1 *Study region and sampling design.*—In the Philippines, over 570 species of migrant and resident birds are distributed across a tropical archipelago stretching latitudinally along the Western Pacific Rim (Kennedy et al. 2000). Over 7,000 islands make up the Philippines, and many islands host their own endemic species and subspecies (Dickinson et al. 1991). Avian colonization and vicariance events in the Philippines are likely to have occurred multiple times and from multiple sources (Diamond and Gilpin 1983, Jones and Kennedy 2008), and heavily restricted gene flow is common between at least some island populations (Jones and Kennedy 2008, Oliveros and Moyle 2010, Chapter 1). Unlike

many mainland taxa, species ranges in the Philippines are likely to have been quite stable over time, having experienced little Pleistocene fluctuation (Jones and Kennedy 2008).

For genetic data, we sampled 1-11 individuals per population from 136 populations, including 118 described subspecies of 50 Philippine bird species representing 31 families and 12 orders (Table S2.1). Population sample sizes greater than n = 2 occur here through use of data from another study (Chapter 1). Multiple comparisons were made in some species, and 152 pairwise genetic comparisons were performed. Of these, 122 comparisons represent 102 populations (including 35 species and 85 subspecies) of perching birds, or Passeriformes (Table S2.1).

2.3.2 *Phenotypic comparisons.*—Phenotypic data were obtained for a subset of the populations for which genetic data were obtained. We performed 63 pairwise phenotypic comparisons of 96 populations (including 93 subspecies) from 43 species (29 families and 12 orders). We followed the Tobias et al. (2010) quantitative method for scoring plumage and biometric characters, wherein *minor*, *moderate*, *major*, and *exceptional* differences in plumage received scores of 1, 2, 3, and 4, respectively, and differences among biometric characters were calculated as Cohen's *d* effect sizes (the difference between means divided by standard deviation) for wing chord, tail length, tarsus length, and bill length between populations and scored as follows: 0.2-2 = *minor*, 2-5 = *moderate*, 5-10 = *major*, and >10 = *exceptional*. Biometric comparisons were performed among samples of n = 2 with equal sex ratios for all species. Immature birds were not included in plumage or biometric analyses. We were unable to follow the

recommendation from Tobias et al. (2010) to include vocal scores, as sufficient data were not available for most populations, nor did we include scores for the presence or absence of hybrid zones, because most of the populations we studied are strictly allopatric. Because selection acts non-independently on biometric characters more commonly than on plumage characters, each phenotypic pairwise comparison presented here considers the three highest scores for plumage and the only greatest increase and decrease in biometric effect sizes between populations. Tobias et al. (2010) recommended that total scores of seven or more should be regarded as sufficiently divergent to be considered as full species. Despite the exclusion of vocal scoring and geographic structure, we also consider a phenotypic score of seven, conservatively, to be a threshold for identifying what we term phenotypically highly divergent taxa.

2.3.3 *Genotypic comparisons*.—DNA was extracted from fresh frozen tissues (or temporarily ethanol-preserved tissues for borrowed samples) from 534 individuals from 50 species of Philippine birds using Qiagen DNeasy blood and tissue extraction kits. The mitochondrial gene ND2 was amplified using standard PCR protocols with the forward primer L5215 (Hackett 1996) and reverse primer H6313 (Johnson and Sorenson 1998). Sanger sequencing was performed by the University of Washington's High Throughput Genomics lab (www.htseq.org) using the same primers and the following custom internal forward primers designed for this study; Eumy.ND2inter (ACAAAAACCCCAGCACTWAG), Hypsi.ND2inter (TAAACTCAATCAAAACCCTA), OtusND2inter (CCCAACCCTATTGACCMYAA),

ParusND2inter (TTCTCCTCCATCTCCCACCT), Phapitre.ND2inter (CTACTAACCTTCTAYCTWTA), SittaND2inter (TATTAACCACCATAGCCATC), and Zoster.ND2inter (CTACTCACATGCATAGCCGT), Collo.ND2inter (TCCCATCTCGGATGAATATC), Microhi.ND2inter (ATAATAATTACCTGAACAAA), and Phyllos.ND2inter (ACCGGRCTRCTMCTRTCCACA). Sequence data were visually inspected and cleaned when required then aligned using Sequencher 4.0. Partial ND2 sequences ranged in length from 428 to 1040 bp (avg. = 944 bp). Genetic divergence was calculated as Jukes-Cantor corrected *p*-distance in MEGA 5.0 (Tamura et al. 2011). MtDNA (ND2) haplotype networks were generated for 8 species in DnaSP and Network 4.6.1.0 (Bandelt et al. 1999, Librado and Rozas 2009).

2.3.4 *Divergence levels*.—We do not propose species limit thresholds in this study. Placing thresholds for species limits on genetic data is an inherently contentious issue (Moritz and Cicerro 2004, Winker 2009), and here we have chosen a rather high threshold (5%) to be conservative in binning what we term genetically highly divergent lineages. While our intention is not to ignore populations with less than 5% genetic divergence, we have chosen this conservative threshold to include consideration of the variability of mutation rates among birds, especially at shallow levels of divergence (Nabholz et al. 2009, 2011, Ho 2007, Ho et al. 2011). We thus set a genetic divergence threshold at 5% Jukes-Cantor corrected ND2 *p*-distance, and a phenotypic divergence threshold of a phenotypic score of 7 or greater to categorize levels of divergence among

the species compared. Population pairs were binned accordingly into four categories: *a)* those that are both phenotypically and genetically divergent (above our thresholds), *b)* those that are phenotypically divergent (i.e., a score above 7) but not genetically divergent, *c)* those that are genetically divergent but not phenotypically divergent, and *d)* those populations whose divergence did not cross either conceptual threshold. A fifth category *e)* contains those comparisons for which only genetic data were available that achieved the 5% genetic distance threshold. Genetic data were plotted against phenotypic data for 63 pairwise comparisons.

2.3.5 *Speciation processes.*—We assume that populational divergences (phenotypic and genotypic) are independent between species, and we have a null expectation that these divergence processes will be independent with respect to higher taxonomy. In other words, we expect that divergence between populations within one species is not affected by similar processes occurring within another species, and we use a null model of phylogenetic independence for these processes (e.g., that divergence processes within species are not affected by what order the species is in). Of the 43 species for which both genetic and phenotypic comparisons were performed, 30 are Passeriformes (perching birds) species. We integrated our genetic and phenotypic pairwise comparisons by making a bivariate plot on a speciation process space where genetic and phenotypic divergences represent the two different axes. Multiple pairwise comparisons within some species introduced non-independence in some cases, which would confound synthetic analyses. To correct for this when asking questions about possible higher-order effects,

we averaged all within-species comparisons for species represented by more than two populations. We then used the corrected datasets to perform analyses of variance (ANOVA and MANOVA) and analyses of deviance (ANODEV), to test for the effect of taxonomic order on genetic and phenotypic divergence.

2.4 Results

Genetic distances from 152 comparisons ranged from 0% to 12.7% (ND2 Jukes-Cantor corrected *p*-distance), and average divergence among populations was 3.0% (Table S2.2. Phenotypic scores from 63 comparisons ranged from 1 to 15 and averaged 6.1 (Table 2.1). Plumage characters (scored as 1-4) averaged 1.3 across all populations, and biometric effect sizes averaged 2.8 (Tables S2.3 and S2.4).

Among the 63 pairwise comparisons that included both genetic and phenotypic data, 35 population pairs exhibited divergence levels surpassing our conceptual thresholds either genetically, phenotypically, or both (Table 2.1). Additionally, 12 population pairs for which phenotypic data were not available surpassed the genetic divergence threshold (Table S2.2). In total, populations in 47 comparisons (out of 152) were considered highly divergent by our conceptual thresholds, representing 70 populations (66 described as subspecies) from 28 species of 8 orders. 16 of these species (from 6 orders) are considered endemic to the Philippines by current taxonomy.

Plotting the 63 pairwise comparisons with both genotypic and phenotypic data in bivariate process space (Fig. 2.1) showed populations that were highly divergent along both axes binned together (Table 2.1 a, Fig. 2.1 bin A). This bin included 9 pairwise

comparisons of 16 populations from six species and one species pair, all Passeriformes. Populations in 19 comparisons with phenotypic scores >7 but not surpassing the genetic threshold were binned together (Table 2.1 b, Fig. 2.1 bin B) and included 16 species in 7 orders. Twelve populations in 7 pairwise comparisons, including four passerine species and one owl, did not surpass the phenotypic threshold but were greater than 5% genetically divergent and were also binned together (Table 2.1 c, Fig. 2.1 bin C). Populations in 28 comparisons did not surpass either of our conceptual thresholds and are considered as not highly divergent (Table 2.1 d, Fig. 2.1 bin D). Of the comparisons for which phenotypic data was unavailable, 10 populations from 4 species surpassed genetic distances of 5% (Table 2.1 e).

In addition to identifying large divergences within species that may merit taxonomic revison, paraphyletic relationships revealed cryptic populations within at least 4 subspecies, *Accipiter virgatus confusus, Phapitreron leucotis brevirostris, Zosterops montanus vulcani,* and *Copsychus mindanensis mindanensis* (Fig. 2.2). Genetic comparisons revealed mtDNA paraphly at the subspecific level in eight species altogether (Fig. 2.2). Genetic comparisons within the remaining 42 species revealed reciprocally monophyletic subspecies with no gene flow between populations.

2.4.1 *Speciation processes.*—After correcting for non-independence in our phenotypic and genetic comparisons (Table S2.5), analyses of variance (ANOVA and MANOVA) did not indicate significant relationships between taxonomic order and either phenotypic or genetic divergence (Appendix 2.1 A, B). However, under a generalized linear model a

two-way analysis of deviance (ANODEV) indicated a significant taxonomic effect on phenotypic divergence ($LR\ \chi^2 = 21.4$, $P = 0.029$) (Appendix 2.1 A). Because many taxonomic orders were represented only by one species, we performed additional analyses treating all non-Passeriformes species as a single group. Ordinary least squares regressions within each group indicated that the slope of the relationship between genetic and phenotypic divergence was significantly different from zero for Passeriformes ($P < 0.01$), but not for non-Passeriformes ($P = 0.72$; Fig. S2.1 C, D). In considering how populations have diverged in speciation process space, it was apparent that no single route to speciation seems to dominate in this system (Figs. 2.1, 2.3).

2.5 Discussion

2.5.1 *Highly divergent lineages*.— Over half of the species in this study included at least one endemic population significantly divergent enough to be considered as full species by some conceptual standards. The overall high degree of within-species phenotypic and genetic divergence shown by our data supports predictions by Lohman et al. (2010) that avian diversity and endemism are gravely underestimated in the Philippines. Average genetic distance from 152 within-species comparisons was 3%, and distances less than this separate many sympatric species in well-studied mainland systems (Hebert et al. 2004, Winker 2009). Although it is not our intention to define species or recommend species elevations here, we believe we have identified many populations that, upon further investigation, may prove to be full biological species (see Appendix 2.2 for a description of some of the more immediate taxonomic implications of this study).

Populations that were both genetically and phenotypically highly divergent (Table 2.1 a, Fig. 2.1 A) are the most likely candidates for species elevation. Large genetic distances suggest long periods of evolutionary isolation, and large phenotypic differences indicate divergent selection. The 16 populations in this group therefore represent independent evolutionary trajectories, and we consider that further study will likely find that this group contains many unrecognized biological species. For example, we found large divergences between three subspecies within the *Dicrurus hottentottus* complex, a complex that includes many contentious subspecies and putative species (e.g. Allen 2006, Collar 2011). In another case, the sister species pair included in this group, *Ficedula crypta* and *F. dispota* (treated as two subspecies of a single species for the purposes of this study), were previously treated as subspecies of *F. crypta* (DuPont 1971, Dickinson et al. 1991) before later being recognized as separate species (Dickinson et al. 2004). Our results support this split.

Population pairs that were highly divergent phenotypically, but not genetically (Table 2.1 b, Fig. 2.1 bin B), represented 35 populations from 16 species that may be undergoing divergent selection. Given sufficient time under divergent selection, adaptation to local environments may result in reproductive isolation (Coyne and Orr 2004). On the other hand, selection is difficult to disambiguate from phenotypic plasticity (Ghalambor et al. 2007), which may affect our results. Among the 16 species in this group, three represent within-island comparisons: *Dasylophus superciliosus*, *Sitta oenochlamys*, and *Prionochilus olivaceus*. Gene flow and/or incomplete lineage sorting may explain low levels of genetic distance between these populations. There were no

within-island comparisons with high levels of genetic divergence. Although the species in this group (Fig. 2.1 bin B) did not exceed our genetic threshold of 5%, relatively large distances (e.g. 3-4%) separated many populations (Table 2.1 b). This suggests that many of the populations in this group experience also little gene flow and may be en route to speciation. Further corroboration will be needed to propose taxonomic revision. Our results are consistent with phenotypic assessments of *Penelopides panini* by Kemp (1988) and of *Chrysocolaptes lucidus* by Collar (2011), in which phenotypic differences indicated species-level divergence.

Populations that were not phenotypically divergent, but were separated by greater than 5% genetic distance (Fig. 2.1 bin C), included four passerine species and one owl (Table 2.1 c). The divergences observed here likely result from isolation, as changes in mtDNA sequence data are usually interpreted to be neutral or nearly neutral (Avise 1988, Ball and Avise 1992, but see Galtier et al. 2009). However, canalization of phenotypic characters, notably plumage color and pattern, and body size and shape, may contribute to the lack of phenotypic divergence observed between populations in this category (Winker 2009). On the other hand, our measures of phenotype do not include behavior, and bird song is likely to play a role in divergence for many of the species we studied (Gill 2007). The twelve subspecies here (Table 2.1 c) represent cryptically diverse populations, most of which likely merit elevation to full biological species, although further corroboration is needed. Additionally, cryptic populations identified within subspecies from ND2 haplotype data (e.g., *P. leucotis, P. elegans*, and *Z. montanus*, Fig. 2.2) may merit subspecific recognition.

2.5.2 *Speciation processes*.—Comparisons among 43 threshold-crossing pairwise comparisons were distributed across the three routes of the speciation process space (Fig. 2.3) with predominance for Passeriformes species in routes ‘a’ and ‘c’ (or bins A and C in Fig. 2.1). Statistical analyses recovered mixed support for a higher-order taxonomic effect on phenotypic divergence, with support for an ordinal effect under a generalized linear model. Small sample sizes in non-passerine orders may have affected these results. There was no statistical support for a taxonomic effect on genetic divergence, despite the majority of highly genetic divergent taxa being Passeriformes. This is not surprising, however, considering the number of very low genetic distances observed within both passerine and non-passerine species (Table S2.2). In non-passerine species, high levels of phenotypic divergence were seven times more common than high levels of genetic divergence, based on our conceptual thresholds (Fig. 2.1). Regressions suggested that there may be differences in divergence processes between passerines and non-passerines (Fig. S2.1 C, D). However, MANOVA results found no significant overall effect of taxonomy on divergence. Increased sample sizes will be required to establish the basis of the effects, if any, of taxonomy on divergence processes. Reasons behind such effects would likely be complicated, however. Rates of mtDNA evolution are known to vary across taxa (Johnson and Seger 2001, Lovette 2004) and may be faster in Passeriformes than in other birds (Nabholz et al. 2009, 2011). Large phenotypic divergence, likely the result of divergent selection, occurs commonly in both passerines and non-passerines (Fig. 2.1). However, for many birds, especially passerines, song can be more important

for mate selection than plumage (Gill 2007). Because we did not measure vocalizations, phenotypic divergence in songbirds is likely underestimated here in the context of Tobias et al.'s (2010) quantification method. Although we found some support for heterogeneity within the speciation process space considered, overall our data suggest a relatively even dispersion of genetic and phenotypic divergence among different taxa. In other words, among Philippine birds there does not seem to be a major single route to speciation occurring in this process space.

2.5.3 *Taxonomic implications for conservation.*—We found that at least 25 and probably more than 40 taxa currently recognized as endemic subspecies will require elevation to full, biological species status. Our results support the suggestion of Lohman et al. (2010) that the lower rates of endemism in birds (compared to other vertebrates) in the Philippines may be an artifact of misclassifying distinct island populations of birds as subspecies, rather than species. Lohman et al. (2010) also predicted that, upon further investigation, a more accurate measure of endemism among birds in the Philippines may exceed 50%, as other terrestrial vertebrates do. With highly divergent populations within more than half the species we studied, our data provide empirical support for this prediction. Our data demonstrate that cryptic species remain within many currently recognized species of Philippine birds. Taxonomic revision, taking into consideration multiple modes of comparison (e.g., genomic comparisons, phenotypic scoring, behavioral and ecological traits, etc.) is needed. Uniting phenotypic and genetic datasets, as we have done here, will prove essential to such revisions. Taxonomic designation can have real-world consequences on the conservation of populations (Ong et al. 2002), and

in the Philippines birds and other wildlife are severely threatened by anthropogenic forces (Heaney and Regalado 1998, Donald et al. 2010). The results of this study emphasize the need for a reappraisal of Philippine avian diversity expressed by Peterson (2006) and Lohman et al. (2010), and further research on 70 populations within 28 species that appear to be largely through the speciation process.

2.6 Acknowledgments

Our field effort was supported by the U. S. Department of Agriculture, the late Dr. John DuPont, the Eurasian Pacific Wildlife Conservation Foundation, and the Friends of Ornithology. We thank the Department of Environment and Natural Resources of the Philippines (DENR), including the Protected Areas and Wildlife Bureau and the Protected Areas and Wildlife Divisions from Region 7 for permitting and project assistance. We also thank Director Pollisco, Director Mundita Lim, Carlo Custodio, Anson Tagtag, Director Augustus Momongan, Reynaldo Yray, Division chief Kho, Gloria Dawson, and many others who participated in this project. Special thanks also go to our collaborators at the National Museum of the Philippines, including Virgilio Palpal-latoc, Rolly Urriza, and Directors Gabriel Casal and Corazon Alvina, and to Edwin Cedella, Almeo Bontigao, and many others for their invaluable assistance in the field. We thank Herman Mays of the Cincinnati Museum of Natural History and Bob Kennedy for lending tissue samples. Lastly, we thank Tom Braile for his assistance in setting up field efforts.

2.7 Literature Cited

Allen, D. 2006. New records and other observations of birds on the island of Tablas, Romblon province, Philippines. Forktail 22: 77-84.

Avise, J. C., Ball, R. M., and Arnold, J. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Molecular Biology and Evolution 5: 331-344.

Avise, J. C. 1994. Molecular markers: natural history and evolution. Springer.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press.

Ball Jr, R. M., & Avise, J. C. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. The Auk 109: 626-636.

Bandelt, H. J., Forster, P., Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37-48.

Bickford, D., D. Lohman, N. S. Sodhi, P. K. L. Ng, R. Meier, K. Winker, K. Ingram, and I. Das. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution 22:148-155.

Bradshaw, C. J., Sodhi, N. S., and Brook, B. W. 2008. Tropical turmoil: a biodiversity tragedy in progress. Frontiers in Ecology and the Environment 7: 79-87.

Collar, N. J. 2007. Philippine bird taxonomy and conservation: a commentary on Peterson (2006). Bird Conservation International 17: 103-113.

Collar, N. J. 2011. Species limits in some Philippine birds including the Greater Flameback *Chrysocolaptes lucidus.* Forktail 27: 29-38.

Conservation International (Lead Author), McGinley M (Topic Editor). 2008. Biological diversity in the Philippines. *in* Cleveland CJ (ed) Encyclopedia of Earth. Environmental Information Coalition, National Council for Science and the Environment, Washington, DC. [Online.] Available at www.eoearth.org/article/Biological_diversity_in_the_Philippines.

Coyne, J. A., and Orr, H. A. 2004. Speciation. Sunderland, MA: Sinauer Associates.

Diamond, J.M., and Gilpin, M.E. 1983. Biogeographic umbilici and the origin of the Philippine avifauna. Oikos 41:307-321.

Dickinson, E. C., Kennedy, R. S., and Parkes, K. C. 1991. The birds of the Philippines: an annotated check-list (Vol. 12). Tring: British Ornithologists' Union.

Dickinson, E. C., Bahr, N., Dowsett, R., Pearson, D., Remsen, V., Roselaar, C. S., and Schodde, D. 2004. The Howard and Moore Complete Checklist of Birds of the World.

Donald, P., Collar, N., Marsden, S., and Pain, D. 2010. Facing extinction: the world's rarest birds and the race to save them. Poyser, London.

DuPont, J. E. 1971. Philippine birds. Delaware Museum of Natural History. Greenville.

Frost, D. R. 2009. Amphibian species of the World: an online reference. Version 5.3 American Museum of Natural History, New York. [Online.] Available at: http://research.amnh.org/vz/herpetology/amphibia/.

Galtier, N., Nabholz, B., Glémin, S., and Hurst, G. D. D. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology 18: 4541-4550.

Ghalambor, C. K., McKay, J. K., Carroll, S. P., and Reznick, D. N. 2007. Adaptive versus non‐adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21: 394-407.

Gill, F. B. 2007. Ornithology. Macmillan Publishers.

Heaney, L.R., and Regalado, Jr., J.C. 1998. Vanishing treasures of the Philippine rainforest. The Field Museum, Chicago.

Hebert P.D.N., Stoeckle M.Y., Zemlak T.S., and Francis C.M. 2004. Identification of Birds through DNA Barcodes. PLoS Biol 2: e312. doi:10.1371/journal.pbio.0020312

Ho, S. Y. 2007. Calibrating molecular estimates of substitution rates and divergence times in birds. Journal of Avian Biology 38: 409-414.

Ho, S. Y., Lanfear, R., Bromham, L., Phillips, M. J., Soubrier, J., Rodrigo, A. G., and Cooper, A. 2011. Time‐dependent rates of molecular evolution. Molecular Ecology 20: 3087-3101.

Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52: 577-586.

Johnson, K. P., and Seger, J. 2001. Elevated rates of nonsynonymous substitution in island birds. Molecular Biology and Evolution 18: 874-881.

Johnson, K. P. & Sorenson, G. D. 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome b and ND2) in the dabbling ducks (tribe: Anatini). Molecular Phylogenetics and Evolution 10, 82-94.

Jones, A. and Kennedy, R. 2008. Evolution in a tropical archipelago: comparative phylogeography of Philippine fauna and flora reveals complex patterns of colonization and diversification. Biological Journal of the Linnean Society 95: 620-639.

Joppa, L. N., Roberts, D. L., Myers, N., and Pimm, S. L. 2011. Biodiversity hotspots house most undiscovered plant species. Proceedings of the National Academy of Sciences 108: 13171-13176.

Kemp, A. C. 1988. The systematics and zoogeography of Oriental and Australasian hornbills (Aves: Bucerotidae). Bonn. Zool. Beitr, 39: 315-345.

Kennedy, R., Gonzalez, P., Dickinson, E., Miranda, H. Jr., and Fisher, T. 2000. A guide to the birds of the Philippines. Oxford University Press.

Librado, P. and Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452

Lohman, D. J., Ingram, K. K., Prawiradilaga, D. M., Winker, K., Sheldon, F. H., Moyle, R. G., Ng, P. K. L., Ong, P. S., Wang, L. K., Braile, T. M., Astuti, D., and Meier, R. 2010. Cryptic diversity in "widespread" southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. Biological Conservation 143: 1885-1890.

Lovette, I. J. 2004. Mitochondrial dating and mixed support for the "2% rule" in birds. The Auk 121: 1-6.

MacArthur, R. H., and Wilson, E. O. 1967. The theory of island biogeography (Vol. 1). Princeton University Press.

Mayr, E. 1963. Animal species and evolution. Belknap Press.

Moritz, C., and Cicero, C. 2004. DNA barcoding: promise and pitfalls. PLoS biology 2: 1529-1529.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G.A.B., and Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853–858.

Nabholz, B., Glémin, S., and Galtier, N. 2009. The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. BMC Evolutionary Biology 9: 54.

Nabholz, B., Künstner, A., Wang, R., Jarvis, E. D., and Ellegren, H. 2011. Dynamic evolution of base composition: causes and consequences in avian phylogenomics. Molecular Biology and Evolution 28: 2197-2210.

Network 4.6.1.0., released 30 December 2011, expiry date 1 January 2013. Network. Copyright Fluxus Technology Ltd. 1999-2012. www.fluxusengineering.com.

Oliveros, C. H., and Moyle, R. G. 2010. Origin and diversification of Philippine bulbuls. Molecular Phylogenetics and Evolution 54: 822–832.

Ong, P. S., Afuang, L. E., and Rosell-Ambal, R. G. 2002. Philippine biodiversity conservation priorities: A second iteration of the National Biodiversity Strategy and Action Plan. DENR-Protected Areas and Wildlife Bureau, CI-Philippines, UP CIDS and FPE, Quezon City Philippines.

Price, T. 2008. Speciation in birds. Roberts & Company Publishers.

Peterson, A. T. 2006. Taxonomy is important in conservation: a preliminary reassessment of Philippine species-level bird taxonomy. Bird Conservation International 16: 155-173.

Rasmussen, P. C., Allen, D. N. S., Collar, N. J., Demeulemeester, B., Hutchinson, R. O., Jakosalem, P. G. C., Kennedy, R. S., Lambert, F. R., and Paguntalan, L. M. 2012. Vocal divergence and new species in the Philippine Hawk Owl *Ninox philippensis* complex. Forktail 28: 1-20.

Sargis, E. J., Campbell, K. K., and Olson, L. E. 2013. Taxonomic Boundaries and Craniometric Variation in the Treeshrews (Scandentia, Tupaiidae) from the Palawan Faunal Region. Journal of Mammalian Evolution DOI 10.1007/s10914-013-9229-2.

Stevens, G. C. 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. American Naturalist 133: 240-256.

Tobias, J. A., Seddon, N., Spottiswoode, C. N., Pilgrim, J. D., Fishpool, L. D., and Collar, N. J. 2010. Quantitative criteria for species delimitation. Ibis 152: 724-746.

Wilson, D. E., and Reeder, D. M. (Eds.). 2005. Mammal species of the world: a taxonomic and geographic reference (Vol. 2). Johns Hopkins University Press.

Winker, K. 2009. Reuniting genotype and phenotype in biodiversity research. BioScience 59: 657-665.

Winker, K., and S. M. Haig (eds.). 2010. Avian subspecies. Ornithological Monographs 67: i -viii, 1-200.

Table 2.1. Genetic distances (Jukes-Cantor corrected *p*–distance) and phenotypic scores (based on the Tobias *et al.* (2010) method) for 63 pairwise comparisons. Results are binned into five categories: *a*) populations diverging across both genetic and phenotypic conceptual thresholds, *b*) high phenotypic divergence, *c*) high genetic divergence, *d*) populations that did not show divergence levels above conceptual thresholds, and *e)* populations with high genetic divergence for which phenotypic data is unavailable.

	Population 1	**Population 2**	**Genetic Distance**	**Phenotypic Score**
a)	Populations diverging across both genetic and phenotypic conceptual thresholds.			
	Dicrurus hottentottus striatus	*D. h. palawanensis*	**6.43%**	**9**
	D. h. palawanensis	*D. h. samarensis*	**5.96%**	**9**
	Orthotomus castaneiceps chloronotus	*O. c. rabori*	**10.61%**	**8**
	Sitta oenochlamys apo	*S. o. isarog*	**5.54%**	**7**
	S. o. apo	*S. o. mesoleuca*	**5.61%**	**10**
	Ficedula disposita	*F. crypta*	**11.36%**	**11**
	Eumyias panayensis nigrimentalis	*E. p. nigriloris*	**5.15%**	**12**
	Prionochilus olivaceus parsonsi	*P. o. olivaceus*	**5.07%**	**7**
	Anthreptes malacensis birgitae	*A. m. paraguae*	**9.46%**	**8**
b)	Populations with high phenotypic divergence.			
	Phapitreron leucotis leucotis	*P. l. nigrorum*	**2.04%**	**9**
	P. l. leucotis	*P. l. brevirostrus*	**3.01%**	**8**
	Dasylophus superciliosus superciliosus	*D. s. cagayensis*	**1.64%**	**11**
	Halycon coromanda linae	*H. c. major*	**2.09%**	**9**
	Penelopides panini panini	*P. p. affinis*	**2.63%**	**12**
	Buceros hydrocorax hydrocorax	*B. h. semigaleatus*	**1.38%**	**9***
	Chrysocolaptes lucidus haemitribon	*C. l. montanus*	**3.43%**	**15**
	Collocalia esculenta marginata	*C. e. bagobo*	**2.81%**	**8**
	Pitta erythrogaster erythrogaster	*P. e. thompsoni*	**0.07%**	**7**
	Pitta sordida sordida	*P. s. palawanus*	**2.34%**	**8**
	Coracina striata striata	*C. s. difficilis*	**1.65%**	**12**
	Pycnonotus urostictus urostictus	*P. u. atricaudatus*	**4.45%**	**7**
	P. u. atricaudatus	*P. u. ilokensis*	**4.23%**	**8**
	P. u. philippensis	*P. u. ilokensis*	**2.97%**	**7**
	Phylloscopus cebuensis cebuensis	*P. c. luzonensis*	**2.29%**	**7**
	Sitta oenochlamys isarog	*S. o. mesoleuca*	**0.21%**	**8**
	Ficedula hyperythra dulangana	*F. h. montigena*	**2.49%**	**7**
	Prionochilus olivaceus parsonsi (Apayao)	*P. o. parsonsi* (Aurora)	**0.10%**	**9**
	Dicaeum hypoleucum pontifex	*D. h. cagayensis*	**3.28%**	**8**
c)	Populations with high genetic divergence.			
	Otus megalotis megalotis	*O. m. everetti*	**5.20%**	**4**
	Rhipidura cyaniceps cyaniceps	*R. c. albiventris*	**5.17%**	**6**
	Corvus enca sierramadrensis	*C. e. pusillus*	**7.18%**	**5**
	Pycnonotus goiavier samarensis	*P. g. suluensis*	**6.49%**	**2**
	P. g. suluensis	*P. g. goiavier*	**6.38%**	**4**
	Ixos philippinus philippinus	*I. p. guimarensis*	**12.42%**	**3**
	I. p. saturatior	*I. p. guimarensis*	**12.34%**	**5**

Table 2.1 continued…

d)	Populations that did not show divergence levels above conceptual thresholds.			
	Gallus gallus philippensis (Bohol)	*G. g. philippensis* (Luzon)	**0.00%**	**3**
	Microhierax erythrogenys erythrogenys	*M. e. maridonalis*	**4.19%**	**6**
	Accipiter virgatus confusus	*A. v. quagga*	**0.53%**	**4**
	Phapitreron leucotis brevirostris	*P. l. nigrorum*	**1.64%**	**5**
	Ninox philippensis philippensis	*N. p. centralis*	**3.03%**	**3**
	Harpactes ardens ardens	*H. a. herbeti*	**2.08%**	**6**
	Gerygone sulphurea simplex	*G. s. rhizophorae*	**0.30%**	**4**
	Pachycephala philippinensis philippensis	*P. p. apoensis*	**3.78%**	**6**
	Pachycephala albiventris albiventris	*P. a. crissalis*	**0.63%**	**2**
	Dicrurus hottentottus striatus	*D. h. samarensis*	**0.63%**	**5**
	Dicrurus balicassius balicassius	*D. b. abraensis*	**1.34%**	**5**
	Parus elegans elegans	*P. e. giliardi*	**0.53%**	**1**
	P. e. elegans	*P. e. montigenus*	**0.47%**	**4**
	P. e. giliardi	*P. e. montigenus*	**0.18%**	**4**
	Pycnonotus urostictus urostictus	*P. u. philippensis*	**3.19%**	**5**
	P. u. atricaudatus	*P. u. philippensis*	**4.67%**	**3**
	P. u. urostictus	*P. u. ilokensis*	**0.21%**	**2**
	Pycnonotus goiavier samarensis	*P. g. goiavier*	**0.43%**	**4**
	Ixos philippinus philippinus	*I. p. saturatior*	**0.98%**	**6**
	Sarcops calvus melanonotus	*S. c. calvus*	**0.32%**	**1**
	Copyschus mindanensis mindanensis	*C. m. deuteronymus*	**0.20%**	**1**
	Cyornis rufigastra philippinensis	*C. r. blythi*	**0.51%**	**3**
	Dicaeum trigonostigma cinereigulare	*D. t. xanthopygium*	**4.39%**	**6**
	Dicaeum australe (Luzon)	*D. australe* (Mindanao)	**0.39%**	**3**
	Cinnyris jugularis jugularis	*C. j. aurora*	**4.51%**	**3**
	C. j. jugularis	*C. j. obsurior*	**0.72%**	**3**
	C. j. aurora	*C. j. obsurior*	**4.81%**	**5**
	Lonchura leucogastra manueli	*L. l. everetti*	**0.25%**	**2**
e)	Populations with high genetic divergence for which phenotypic data is unavailable			
	Ninox philippensis philippensis	*N. p. spilocephala*	**5.08%**	**n/a**
	N. p. centralis	*N. p. spilocephala*	**5.72%**	**n/a**
	Irena cyanogaster cyanogaster	*I. c. hoogstraali*	**6.22%**	**n/a**
	Sitta oenochlamys oenochlamys	*S. o. apo*	**5.23%**	**n/a**
	Eumyias panayensis panayensis	*E. p. nigrimentalis*	**5.05%**	**n/a**
	E. p. panayensis	*E. p. nigriloris*	**5.63%**	**n/a**

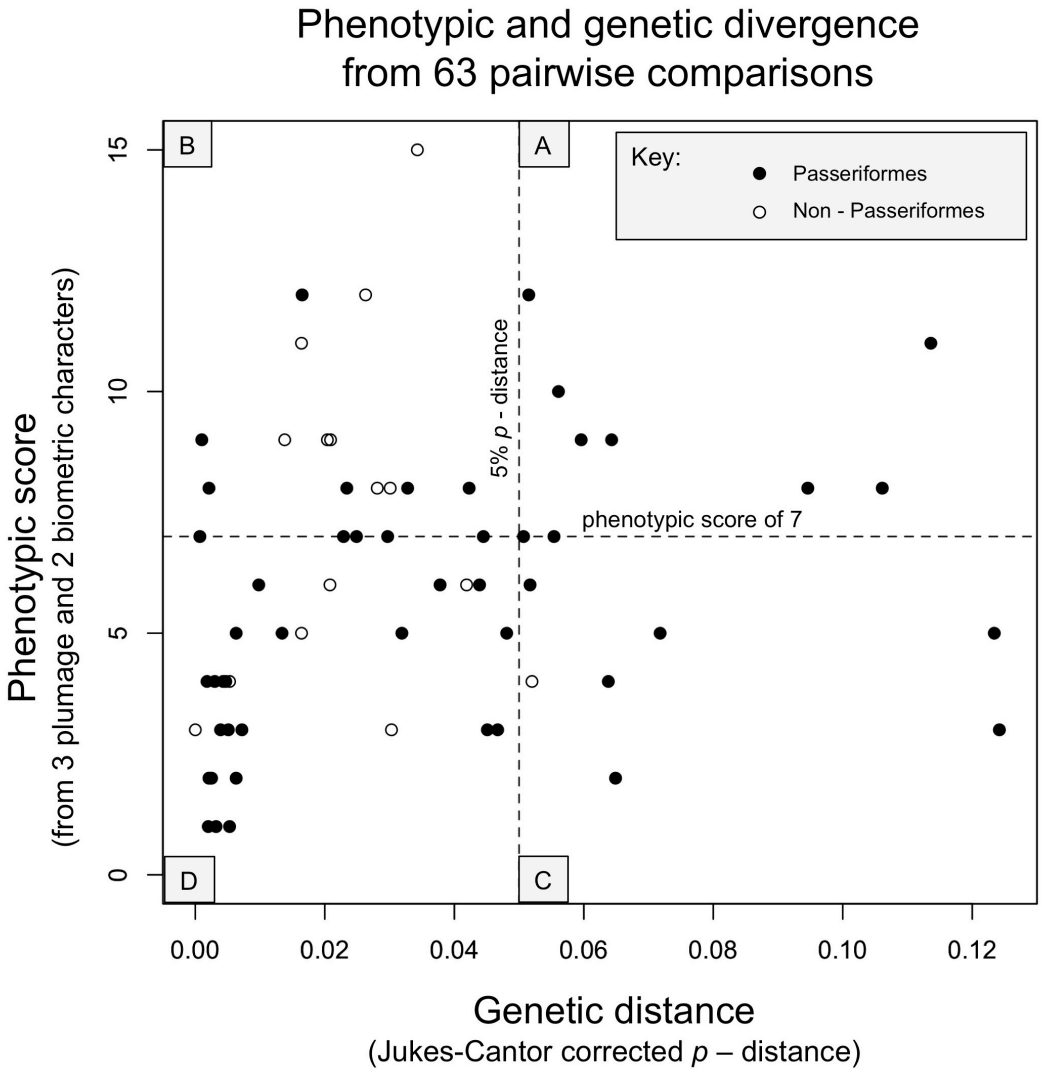


Figure 2.1. Phenotypic divergence plotted against genetic divergence for 63 pairwise comparisons within 43 species. For binning purposes, thresholds of divergence are set at a phenotypic score of 7 and 5% genetic divergence. These thresholds partition the results into 4 bins: A) Populations diverging across both genetic and phenotypic thresholds, B) high phenotypic divergence with low genetic divergence, C) high genetic divergence with low phenotypic divergence, and D) populations that did not surpass thresholds. Open circles represent comparisons among non-Passeriformes taxa, and closed circles represent Passeriformes. An additional 6 pairwise comparisons surpassed the genetic divergence threshold of 5% (Table 2.1 e), however due to lack of phenotypic data they are not included here.

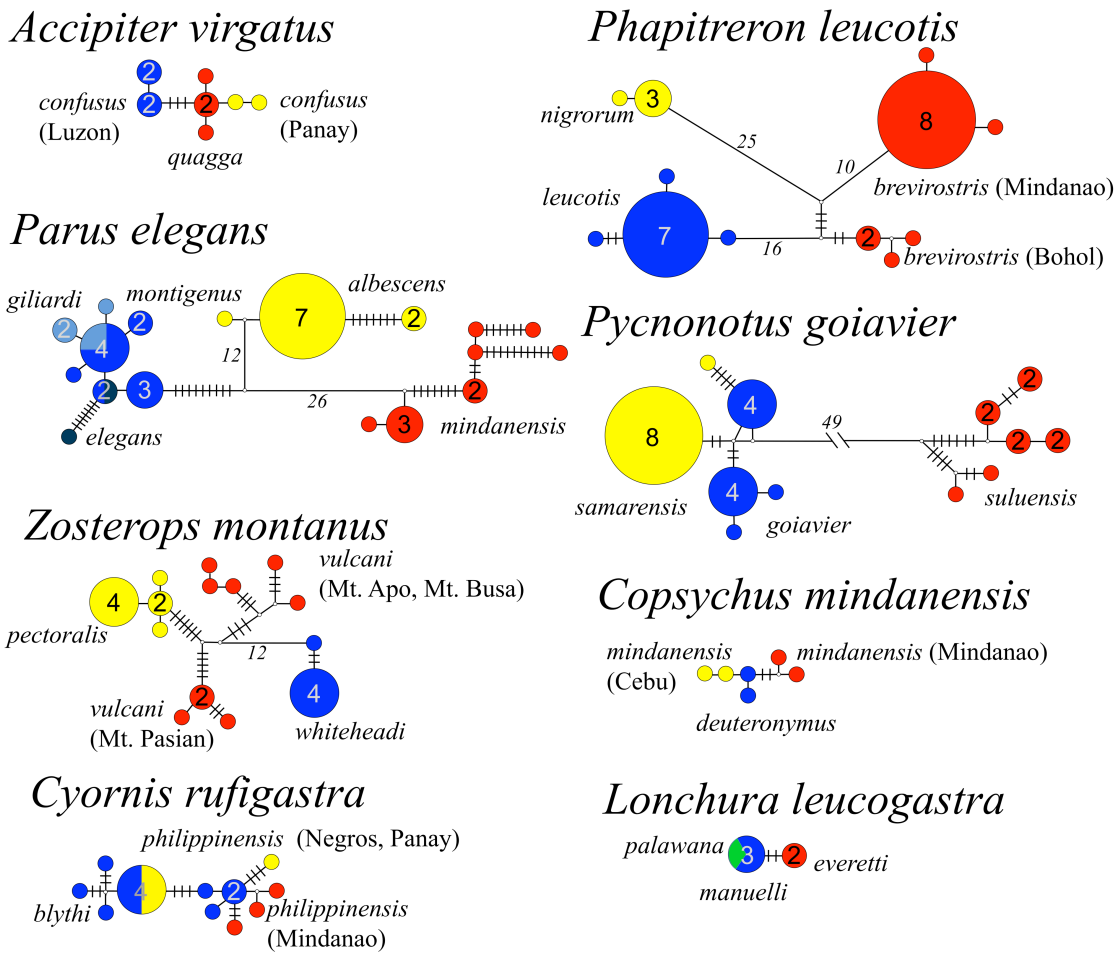


Figure 2.2. Haplotype networks showing mtDNA paraphyly between 14 subspecies of 8 species. The number of individuals per haplotype and the number of mutations separating haplotypes is given for all numbers greater than 1. Numbers printed over haplotypes refer to the number of individuals, and dashes or numbers between haplotypes refer to the number of mutations. Colors correspond to geographic regions: Blue = Luzon, Yellow = Visayas, Red = Mindanao, and Green = Palawan.

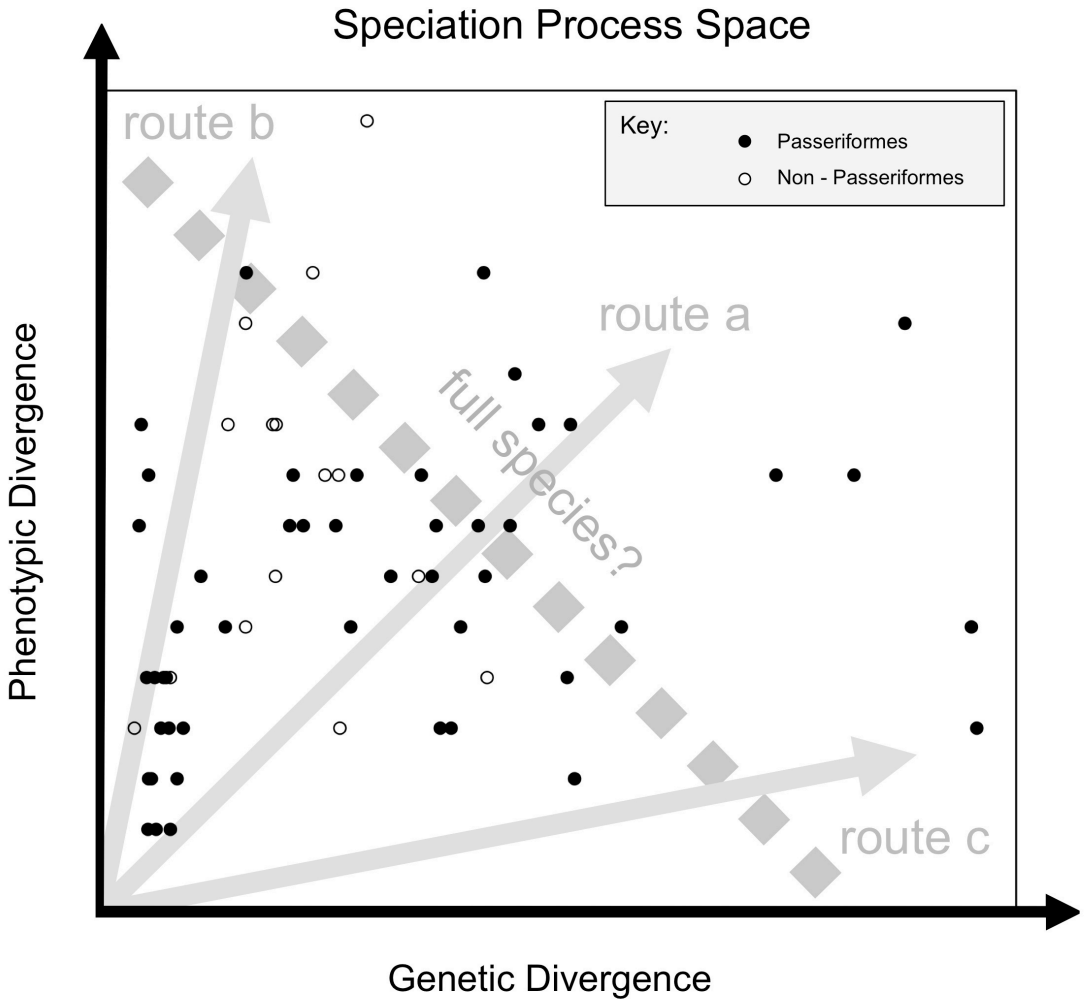


Figure 2.3. Speciation process space showing three routes to speciation along two axes: phenotypic divergence and genetic divergence. Pairwise comparisons from Table 2.1 and Figure 2.1 are plotted over this process space, and the dashed dotted line indicates a hypothetical species threshold.

2.8 Supplementary Materials

Table S2.1. List of all specimens compared in this study including taxon identification, locality, field catalog numbers, museum voucher numbers, and GenBank accession numbers for ND2 sequences generated in this study. Specific locality information is available upon request from the authors.
(see attached supporting materials)

Table S2.2. Genetic distances for all 152 pairwise comparisons in this study. Sample size, ND2 fragment size, and standard deviation of genetic distance is given.
(see attached supporting materials)

Table S2.3. Specific phenotypic differences from all pairwise comparisons. (see attached supporting materials)

Table S2.4. Specific biometric measurements and Cohen's *d* effect size calculator for all pairwise comparisons.
(see attached supporting materials)

Table S2.5. Genetic and phenotypic divergence scores after correcting for non-independence.
(see attached supporting materials)

Appendix A.

A

ANOVA and Analysis of Deviance results

Effect of taxonomic order on phenotypic divergence:
2-way ANOVA:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Order	11	181.30	16.4819	1.9467	0.07133 .
Residuals	31	262.47	8.4667		

2-way Analysis of Deviance under generalized linear model:

	LR Chisq	Df	Pr(>Chisq)
Order	21.413	11	0.02933

Effect of taxonomic order on genetic divergence:
2-way ANOVA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Order	11	30.885	2.8077	0.2889	0.9834
Residuals	31	301.299	9.7193		

2-way Analysis of Deviance under generalized linear model:

	LR Chisq	Df	Pr(>Chisq)
Order	3.1777	11	0.9882

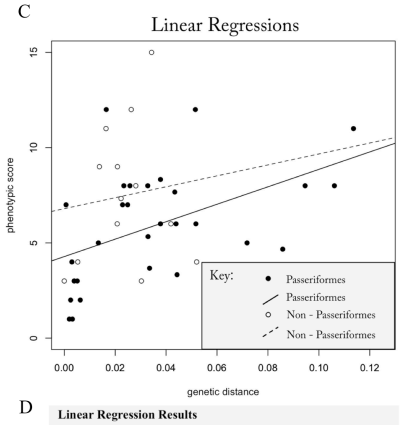
B

MANOVA results

Effect of taxonomic order on both genetic and phenotypic divergence:

	Df	Pillai	approx F num	Df	den Df	Pr(>F)
Order	11	0.58874	1.1757	22	62	0.3016
Residuals	31					

C



D

Linear Regression Results

Phenotypic x Genetic divergence (Non-Passeriformes)
Residuals:
Min 1Q Median 3Q Max
-0.022147 -0.009106 -0.001665 0.007115 0.029433

Coefficients:
Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.0208875 0.0094886 2.201 0.050
yn 0.0004198 0.0011457 0.366 0.721

Residual standard error: 0.01463 on 11 degrees of freedom
Multiple R-squared: 0.01206, Adjusted R-squared: -0.07775
F-statistic: 0.1343 on 1 and 11 DF, p-value: 0.721

Phenotypic x Genetic divergence (Passeriformes)
Residuals:
Min 1Q Median 3Q Max
-0.050660 -0.015723 -0.008862 0.010027 0.060153

Coefficients:
Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.003521 0.011604 0.303 0.76384
yp 0.005303 0.001770 2.997 0.00566

Residual standard error: 0.02839 on 28 degrees of freedom
Multiple R-squared: 0.2429, Adjusted R-squared: 0.2158
F-statistic: 8.981 on 1 and 28 DF, p-value: 0.005661

Appendix A. Results of contrasts between Passeriformes and non-Passeriformes species including results of ANOVA and ANODEV (A), MANOVA (B), and Linear Regressions (C and D).

Appendix B. *Taxonomic Implications.*—When comparing a single genetic marker among subspecies, paraphyly is to be expected (Winker 2009, 2010). In this study, the absence of paraphyly in 42 species is more remarkable than its presence in 8 (Fig. 2.2). Much of the subspecific paraphyly displayed in Figure 2 probably results from recurrent gene flow between populations or incomplete lineage sorting. However, divergence within *Zosterops montanus vulcani* is likely cryptic diversity that merits phenotypic examination for a possible new subspecies. *Phapitreron leucotis brevirostris* from Bohol Island may also warrant recognition as its own subspecies. Although corroboration is needed, paraphyly and shallow distances between subspecies in *Lonchura leucogastra*, *Cyornis rufigastra*, and *Parus elegans* indicate populations that may have been over-split. Or, conversely, these may be more typical of avian subspecies in general, in which phenotypic traits enable recognition of populations that are not concordant with mtDNA, such discordance stemming from the different actions of selection and neutral genetic changes.

The distinctiveness of *Dicrurus hottentottus palawanus* from two other Philippine populations (*D. h. striatus* and *D. h. samarensis*; Table 2.1 a) and Collar's (2011) assessment of the phenotypic divergence between *D. h. menagei* from Tablas Island and *D. h. palawanus* suggest that this species complex is in major need of revision. Almost certainly, *D. h. palawanus,* separated from other populations in this study by large genetic and phenotypic divergence (Table 2.1 a) and geographically isolated on Palawan and its associated islands from the oceanic islands of the Philippines, should be elevated to species status.

Sheldon *et al.* (2012) found deep genetic (ND2) divergence between *O. c. castaneiceps* from Panay and *O. c. chloronatus* from Luzon, similar to the divergence we observed between *chloronatus* and *rabori* from Cebu. We suspect *O. c. chloronatus* deserves elevation to a full biological species based on exceptionally high genetic divergence and high phenotypic divergence (Table 2.1 a).

Jones and Kennedy (2008) showed *Eumyias panayensis* subspecies *nigriloris* and *nigrimentalis* to exhibit reciprocal monophyly, and our data suggest that this species may warrant being split into multiple species based on deep genetic and phenotypic divergences (Table 2.1 a, e).

We observed striking phenotypic divergence between two populations of *Prionochilus olivaceus parsonsi* from Luzon Island (Apayao and Aurora Provinces; score = 9, Table 2.1 b). Despite very little genetic divergence, the phenotypic divergence exhibited between these two populations merits further investigation that may result in recognition of two subspecies in northern Luzon.

General Conclusions

The birds of the Philippines represent an excellent window into the dynamic processes of speciation in tropical islands. The Philippines have played a historical role in the development of evolutionary theory and biogeography (Wallace 1858, Huxley 1868) and continue to inform our understanding of evolution on islands (e.g., Jones & Kennedy 2008, Sheldon *et al.* 2009, Chapter 1). In this thesis I compared the distribution of population genetic diversity among Philippine birds with mainland models and found no support for latitudinal or mid-domain effects. I then explored levels of genetic and phenotypic divergence within 50 species and found empirical support for predictions that avian diversity is underestimated in the Philippines. The results of this thesis revealed interesting patterns within two different levels of emergent diversity; population genetic diversity was correlated with an inferred measure of time spent in evolutionary isolation, and there was mixed support for a taxonomic effect on speciation processes among divergent populations.

In my first chapter I measured the population genetic diversity of 28 populations from 9 species and hypothesized that geographic factors including latitude, island size, and/or species range geometry would affect the distribution of population genetic diversity. The species I analyzed did not conform to the models tested, providing support only for a null model of randomly distributed population genetic diversity. This unexpected result led to the discovery of a significant relationship between the inferred amount of time spent in evolutionary isolation and genetic diversity within island populations. This suggests that there is a link in this system between two sets of processes

usually considered separately, the generation of genetic diversity within populations and the generation of new evolutionary lineages (speciation). While further research is needed to better understand this relationship, its occurrence raises questions about the nature of genetic diversity in isolated systems.

Populations in more than half of the 50 species studied in my second chapter represent species-level divergences under conservative criteria of genetic and phenotypic divergence. This provides strong support for suggestions that avian diversity and endemism are underestimated in the Philippines (Lohman *et al.* 2010). At least 25 and as many as 70 island populations in this study represent unique evolutionary trajectories that will likely merit elevation to full biological species upon further investigation. The taxonomic and conservation implications of this research are clearly important.

Speciation is a dynamic process driven by multiple factors, and multiple routes toward speciation exist (Mayr 1964, Price 2008, Winker 2009). Through examination of both genetic and phenotypic divergence, multifarious combinations of both aspects of divergence revealed patterns within a complex process space in which avian populations diverge in the Philippines. Mixed support for an association between taxonomic order and the heterogeneity of divergence observed in this process space requires further study.

Although a complete picture of the speciation processes among Philippine birds has not been presented here, these results provide meaningful progress in our understanding of both divergence within species and the generation of genetic diversity within populations, both of which have significant implications for taxonomy, conservation, and further research.

References

Collar, N.J. (2011). Species limits in some Philippine birds including the Greater Flameback *Chrysocolaptes lucidus. Forktail*, 27, 29-38.

Darwin, C. (1859). *On the origins of species by means of natural selection*. London. Murray.

Diamond, J.M., and Gilpin, M.E. (1983). Biogeographic umbilici and the origin of the Philippine avifauna. *Oikos*, 41, 307-321.

Dickinson, E. C., Kennedy, R. S. & Parkes, K. C. (1991). *The birds of the Philippines: an annotated check-list (Vol. 12).* Tring: British Ornithologists' Union.

Heaney, L.R. & Regalado, Jr., J.C. (1998). *Vanishing treasures of the Philippine rainforest*. The Field Museum, Chicago.

Huxley, T. H. (1868). On the classification and distribution of the Alectoromorphae and the Heteromorphae. *Proc. Zool. Soc. Lond.*, 1868, 294-319.

IUCN. (2013). *The IUCN Red List of Threatened Species*. Version 2013.1. <http://www.iucnredlist.org>. Downloaded on 02 July 2013.

Jones, A. & Kennedy, R. (2008). Evolution in a tropical archipelago: comparative phylogeography of Philippine fauna and flora reveals complex patterns of colonization and diversification. *Biol. Jo. Linn, Soc.*, 95, 620-639.

Kennedy, R., Gonzalez, P., Dickinson, E., Miranda, H. Jr. &Fisher, T. (2000). *A guide to the birds of the Philippines*. Oxford: Oxford University Press.

Lohman, D. J., Ingram, K. K., Prawiradilaga, D. M., Winker, K., Sheldon, F. H., Moyle, R. G., Ng, P. K. L., Ong, P. S., Wang, L. K., Braile, T. M., Astuti, D., and Meier, R. (2010). Cryptic genetic diversity in “widespread” Southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. *Biol. Conserv.*, 143, 1885-1890.

MacArthur, R. H. & Wilson, E. O. (1967). *The theory of island biogeography*, Vol. 1. Princeton University Press.

Mayr, E. (1963). *Animal species and evolution*. Belknap Press, Cambridge, MA.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G.A.B., and Kent J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.

Oliveros, C. H., and Moyle, R. G. (2010). Origin and diversification of Philippine bulbuls. *Mol. Phylog. Evol.,* 54, 822–832.

Peterson, A.T. (2006). Taxonomy is important in conservation: a preliminary reassessment of Philippine species-level bird taxonomy. *Bird Conservation International,* 16, 155-173.

Price, T. (2008). *Speciation in birds*. Greenwood Village, CO. Roberts and Company.

Rasmussen, P. C., Allen, D. N. S., Collar, N. J., Demeulemeester, B., Hutchinson, R. O., Jakosalem, P. G. C., Kennedy, R. S., Lambert, F. R., and Paguntalan, L. M. (2012). Vocal divergence and new species in the Philippine Hawk Owl *Ninox philippensis* complex. *Forktail,* 28, 1-20.

Sheldon, F. H., Oliveros, C. H., Taylor, S. S., McKay, B., Lim, H. C., Rahman, M. A., ... and Moyle, R. G. (2012). Molecular phylogeny and insular biogeography of the lowland tailorbirds of Southeast Asia (Cisticolidae: *Orthotomus*). *Mol. Phylog. Evol.,* 65, 54-63.

TIB Partners. (2012). *Threatened Island Biodiversity Database.* Version 2012.1. <www.tib.islandconservation.org>. Downloaded on 13 June 2013.

Wallace, A. R. (1858). On the tendency of varieties to depart indefinitely from the original type*. J. Proc. Linn. Soc. Zool.*, 3, 53-62.

Winker, K. (2009). Reuniting genotype and phenotype in biodiversity research. *BioScience,* 59, 657-665.